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FACTORS AFFECTING THE SPORULATION OF PHYLLOSTICTA SOLITARIA IN ARTIFICIAL CULTURE

A. J. Mix

(Accepted for publication August 5, 1932)

Several investigators of the apple-blotch fungus, *Phyllosticta solitaria* E. and E., have experienced difficulty in obtaining sporulation in artificial culture. Clinton (3) reports that *P. solitaria* made rather slow growth in culture and "had not, after two months' development, given any sign of the formation of a spore stage." He does not state what medium he used. Scott and Rorer (13) found that pycnidium-like bodies were formed in abundance in all media, but that they usually were sterile. These investigators were able to secure spore-bearing pycnidia only on sterilized apple wood and on corn-meal agar. Lewis (10) obtained spores on apple wood but not on prune agar, potato agar, or potato cylinders. Roberts (12) found that on all ordinary solid culture media *P. solitaria* would form pycnidia but that they were sterile. Pycnidia with spores developed, however, after 2 or 3 months on sterilized apple twigs. Guba (6) had similar difficulty. He found that the fungus produced sterile pycnidia or "pycnosclerotia" on nearly all media employed, but sporulation occurred in his cultures only occasionally. He was unable to induce sporulation by the use of "roll cultures" or by exposing cultures to alternating high and low temperatures. His spore-bearing cultures failed to produce other spore-bearing cultures, and he concludes that there is no evidence of the occurrence of sporulating strains. More recently, Kohl (7) reports the formation of fertile pycnidia on corn-meal agar when the cultures contained the pieces of host tissue used in isolating the fungus. Subcultures from these sporulating cultures failed to produce fertile pycnidia.

Phyllosticta solitaria has been kept in pure culture in this laboratory for several years. One of the earliest observations on its behavior was that a large proportion of the cultures made on various common media produced spores. It seemed worth while, therefore, to study some of the factors that might influence sporulation of this fungus in culture, with the object of finding a medium on which sporulation would occur with some regularity.

INFLUENCE OF LOCALITY

Physiologically distinct strains of many fungi occur in different localities. It seemed possible that herein might lie the explanation of the fact

that *Phyllosticta solitaria* sporulated more readily in this laboratory than elsewhere. Accordingly, in 1925 a culture of the fungus, isolated from Tennessee material, was obtained from C. D. Sherbakoff. At the same time some blotch-cankered apple twigs were obtained from H. W. Anderson, and several isolations were made from them. The Tennessee and Illinois cultures produced spores as freely as did the Kansas cultures. Since as will appear below, there are sporulating and nonsporulating strains of the fungus in one locality, it seemed useless to pursue this point further.

INFLUENCE OF SOURCE OF CULTURE

It seemed possible that there might be some difference in behavior between cultures isolated from different parts of the host. Isolations were therefore made from twig cankers, fruits, and leaves; the fungus was grown on several media, chiefly potato agar without added sugar. The results are summarized below.

Cultures from twigs	Sporulating 73	Nonsporulating 44
" " fruits	" 47	" 33
" " leaves	" 31	" 37

There is clearly no significant difference as to ability to produce spores between cultures obtained from twigs, fruit, or leaves.

INFLUENCE OF TIME OF ISOLATION

Another possibility considered was that the time of year when isolations were made might influence the behavior of the fungus in culture. Guba (6) suggests this possibility. He obtained spores in culture from isolations made in February, and none from isolations made in autumn or early winter. His later attempts to obtain spores from isolations made in early spring were, however, unsuccessful.

There have been in this laboratory, during the past several years, nearly 300 cultures of *Phyllosticta solitaria*, each representing a separate isolation. These isolations were made at all seasons: from twig cankers at intervals throughout the whole year and from fruits and leaves during the spring and summer. The record kept on these cultures shows that several for every isolation-date have produced spores.

INFLUENCE OF LIGHT

Coons (4) found that light was necessary for formation of pycnidia by *Plenodomus fuscomaculans*. According to Coons and Levin (5), Levin (9), and Leonian (8), certain species of the Sphaeropsidales require light for pycnidium formation, while others do not. Guba (6), in experiments with *Phyllosticta solitaria*, found that the formation of sterile pycnidia (pycnosclerotia) was not inhibited by darkness.

The results shown in table 1 indicate that *Phyllosticta solitaria* belongs to the light-indifferent forms. In this experiment, as in those subsequently reported, duplicate cultures of each series were made in 125 cc. Erlenmeyer flasks, 40 cc. of the medium being placed in each flask. One series of cultures was exposed to the light from a window, the other was kept in a photographic dark room. The temperature of the 2 rooms was the same within 2 or 3 degrees throughout the experiment.

TABLE 1.—*Influence of light on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Pycnidia formed in light	Spores formed in light	Pycnidia formed in dark	Spores formed in dark
166	+	+	+	+
168	+	+	0	0
169	+	+	0	0
216	+	+	+	+
217	+	+	+	+
218	+	0	+	0
219	+	+	+	+
229	+	0	+	0
230	+	0	+	0
231	+	0	+	0

The performance of cultures 168 and 169 in this experiment might suggest that there are light-sensitive races of *Phyllosticta solitaria,* but whether any significance should be attached to the failure of these cultures to sporulate in the dark could be determined only by further experimentation.

SPORULATION ON COMMON MEDIA

The fungus has been found to produce spores on such common media as potato agar, oatmeal agar (Clinton's formula), corn-meal agar (Shear's formula), Difco corn-meal agar, green-apple agar, string-bean agar, bark-decoction agar, and potato-dextrose agar. The 2 last-named media, in suitable concentration, were found especially favorable to sporulation. They will be given further consideration below in connection with the discussion of quantity of food.

LIQUID VERSUS SOLID MEDIA

Liquid cultures are commonly used in nutrition studies with fungi, especially if quality of food is being considered. It appeared quite early in the present investigation that liquid culture media could not be used.

Cultures were made in various seed broths, potato-dextrose broth, and certain synthetic nutrient solutions. The fungus grew well, forming a considerable amount of submerged mycelium, but formation of pycnidia occurred only after the cultures had dried out enough to allow development of aerial mycelium, and pycnidia were not produced abundantly until the liquid was nearly or quite gone.

An attempt was made to use filter-paper pads in connection with liquid media as suggested by Leonian (8). They were, however, found unsatisfactory with this fungus. An experiment, for example, on quantity of food, in which parallel series of cultures were made using filter-paper pads wet with the nutrient solution and the same solution solidified with agar, resulted in very irregular sporulation on the pads. The agar cultures, on the other hand, gave regular and consistent results. Consequently, in the study of factors yet to be considered agar cultures were employed. There seems to be no objection to the use of agar in such experiments provided the fungus cannot use the agar itself as a source of carbon. Since *Phyllosticta solitaria* makes only negligible growth in cultures where agar supplies the only source of carbon, it can be assumed that agar is not utilized by this fungus.

INFLUENCE OF QUANTITY OF FOOD

The factor that, in these studies, has seemed to have the greatest effect on sporulation is quantity of food. Coons (4), who has studied the effect of food quantity on growth and reproduction of *Plenodomus fuscomaculans* (Sacc.) Coons, has shown that the range of food concentration within which growth of that fungus can take place is wider than that allowing formation of pycnidia and spores. Concentrations of food so low as to be near the subsistence limit did not permit sporulation in his cultures, while an overabundance of food caused excessive mycelial growth with attendant suppression of pycnidia. In his discussion Coons calls attention to the fact that, in most ordinary laboratory media, fungi are greatly overfed.

Phyllosticta solitaria, it will be seen, reacts readily to variations in food concentration. It is quite possible that the failure of previous investigators to obtain sporulation in culture was due to their using media of too high food content. Potato-dextrose agar, as it is ordinarily made in this laboratory (potatoes 140 gm., dextrose 10 gm., agar 20 gm., water 1,000 cc.), appears to be too rich for *P. solitaria*. It will allow sporulation but by no means so readily as more dilute media.

In a rich medium, such as potato-dextrose agar, growth of the fungus is thick and piled up, and separate pycnidia are not visible, but, by their coalescence they form a sclerotium-like crust. Some strains of the fungus form a whitish, aerial mycelium over this crust, others do not. In more

dilute media a radiate growth of appressed mycelium is formed, suggesting a common type of fruit blotch, and separate pycnidia appear throughout the colony. These are quite like the pycnidia formed in fruit blotches and twig cankers and are not like the sterile thick-wall pycnidia referred to by Guba (6) as pycnosclerotia. Photographs of cultures of *Phyllosticta solitaria*, showing types of colony formed in rich and in dilute media, are reproduced in figure 1.

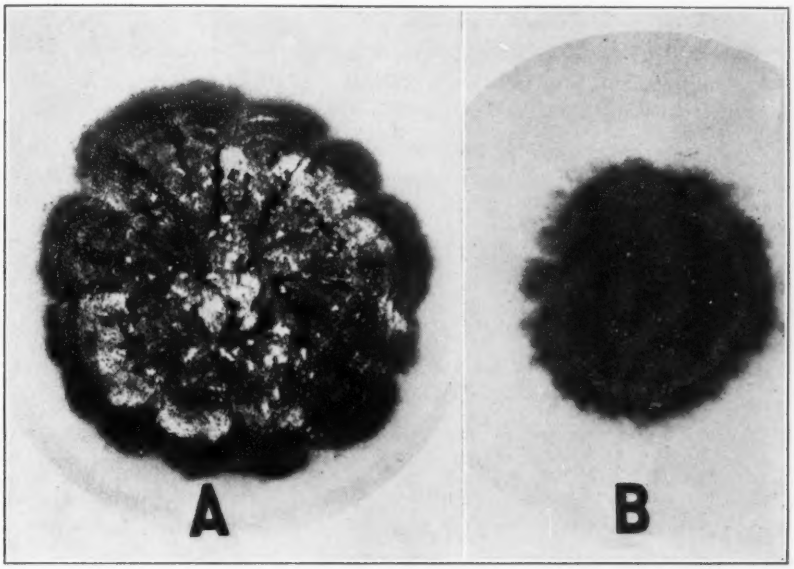

FIG. 1. Colonies of *Phyllosticta solitaria*. A. On S/1 potato-dextrose agar. B. On S/10 potato-dextrose agar. White dots in B are masses of pycnospores oozing from pycnidia.

The results of an experiment on quantity of food are shown in table 2. Potato-dextrose broth of the formula given above was taken as the standard and is designated in the table by the symbol S/1. This standard broth was diluted to $\frac{1}{2}$, $\frac{1}{4}$, etc., its original strength by means of distilled water. These dilutions are shown in the table by the symbols S/2, S/4, etc . All the broths were solidified with 2 per cent agar.

In recording the occurrence of pycnidia it was found convenient to use a system of notation like that of Coons (4), but with slightly different values. In this and the following tables the symbol 0 indicates the absence of pycnidia from a culture; + indicates the presence of from 1 to 25 pycnidia, ++ from 25 to 50; +++ from 50 to 100; ++++ 100 or more. As indicated earlier, all cultures were made in duplicate, each count, therefore, represents the average count of 2 cultures. Where, instead of separate

TABLE 2.—*Effect of different concentrations of potato-dextrose medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration	Pycnidia	Spores	Culture	Concentration	Pycnidia	Spores
Experiment 1							
168	S/1	Cr	+(f)	218	S/64	+++	+
	S/2	Cr	+		S/128	+	+
	S/4	++++	+		S/256	0	0
	S/8	++++	+	219	S/1	Cr	+(f)
	S/16	++++	+		S/2	Cr	+
	S/32	++++	0		S/4	Cr	+
	S/64	0	0		S/8	++++	+
	S/128	0	0		S/16	++++	+
	S/256	0	0		S/32	++++	+
216	S/1	Cr	+		S/64	+++	+
	S/2	Cr	+		S/128	0	0
	S/4	Cr	+		S/256	0	0
	S/8	++++	+	230	S/1	Cr	0
	S/16	++++	+		S/2	Cr	+
	S/32	++++	+		S/4	Cr	+
	S/64	+++	+		S/8	++++	+
	S/128	+	+		S/16	++++	+
	S/256	0	0		S/32	++++	+
217	S/1	Cr	+(f)		S/64	++	+
	S/2	Cr	0		S/128	0	0
	S/4	Cr	+		S/256	0	0
	S/8	++++	+	231	S/1	Cr	+
	S/16	++++	+		S/2	Cr	+
	S/32	++++	+		S/4	Cr	+
	S/64	+++	+		S/8	++++	+
	S/128	0	0		S/16	++++	+
	S/256	0	0		S/32	+++	+
218	S/1	Cr	+(f)		S/64	++	+
	S/2	Cr	+		S/128	0	0
	S/4	Cr	+		S/256	0	0
	S/8	++++	+				
	S/16	++++	+				
	S/32	++++	+				
Experiment 2							
166	8S	Cr	0	232	S/2	++++	+
	4S	Cr	0		S/4	++++	+
	2S	Cr	+(f)		S/8	++++	+
	S/1	Cr	+(f)		S/16	+++	+
	S/2	Cr	0	241	8S	Cr	0
	S/4	++++	+		4S	Cr	+
	S/8	++++	+		2S	Cr	+
	S/16	+	+		S/1	Cr	0
232	8S	Cr	+(f)		S/2	++++	+
	4S	Cr	+		S/4	++++	0
	2S	Cr	+(f)		S/8	++++	+
	S/1	Cr	+		S/16	++++	+

pycnidia, the sclerotial crust described above was formed, that fact is indicated in the tables by replacing the plus marks by the symbol Cr. Presence or absence of spores is indicated by the symbols + and 0, respectively.

In cases where only a few of the pycnidia in a culture bore spores (instead of nearly all the pycnidia, as was the usual case) this fact is indicated by the letter f in parentheses following the plus sign.

A second experiment in which the concentration of potato-dextrose broth was varied between limits of 8 times the usual strength and $\frac{1}{16}$ the usual strength is reported in table 2.

Examination of the results shown in table 2 indicates that the range of food concentration that will allow growth of *Phyllosticta solitaria* in culture is wider than the range for sporulation. As the food concentration is increased beyond that which allows only mycelial growth separate spore-bearing pycnidia occur in the cultures. With further increase in concentration these pycnidia become more numerous until their identity is lost and the attendant increase in mycelial growth results in the formation of the so-called "sclerotial crust." The pycnidia occurring in this crust may bear spores, but further increase in food concentration leads to reduction and even complete inhibition of spore formation.

It was impossible to estimate accurately the difference in amount of mycelial growth in these experiments. Mycelium in the more dilute media is very scanty, appressed, and often partly submerged. In the highest concentrations it is thick and piled up. The difference in volume of mycelium between the extremes is very large and a measurement of the radial spread of the colony would leave this difference out of account. In fact, the only consideration given to mycelial growth in these studies was that necessary to the final choice of a medium that would allow growth sufficiently vigorous to produce a large number of separate spore-bearing pycnidia. For example, it was decided after examination of the cultures in the 2 experiments just described that a convenient concentration of potato-dextrose broth that would allow sufficient growth, formation of separate, scattered pycnidia, and abundant sporulation would be that of $\frac{1}{10}$ the usual strength. Consequently, S/10 potato-dextrose agar has been used in further experimentation whenever a potato-dextrose medium was desired.

A similar effect of the concentration of food was found with apple-bark decoction agar. The bark decoction was prepared by boiling 50 gm. of bark from young apple twigs in a convenient quantity of water for 20 minutes, allowing the water to stand on the bark overnight, then, adding water to the filtered decoction to make a volume of 1,000 cc. The effect of bark decoction in standard strength and in various dilutions on sporulation is shown in table 3. Subsequent use of S/5 bark-decoction agar showed it to be a very favorable medium for the production of separate pycnidia and for sporulation.

An even narrower range of food concentration permitting sporulation was found with Richards' solution modified by substituting dextrose (1%) for the sucrose (5%) of that solution. This modified Richards' solution

TABLE 3.—*Effect of different concentrations of apple-bark decoction and of modified Richards' and Coons' media on pycnidium formation and sporulation of Phyllosticta solitaria*

Apple-bark decoction				Modified Richards' Medium			
Culture	Concentration	Pycnidia	Spores	Culture	Concentration	Pycnidia	Spores
166	S/1	++++	0	241	15S	Cr	0
	S/2	++++	+		10S	Cr	0
	S/4	+++	+		5S	Cr	0
	S/8	++	+		2S	Cr	0
	S/16	+	+		S/1	Cr	0
	S/32	0	0		S/5	++	+
	S/64	0	0		S/15	++	+
232	S/1	++++	0		S/50	0	0
	S/2	++++	+(f)		S/100	0	0
	S/4	++++	+	246	15S	Cr	0
	S/8	++	+		10S	Cr	0
	S/16	+	+		5S	Cr	0
	S/32	0	0		2S	Cr	0
	S/64	0	0		S/1	Cr	0
246	S/1	++++	0		S/5	0	0
	S/2	++++	+(f)		S/15	+	+
	S/4	++	+		S/50	0	0
	S/8	++	+		S/100	0	0
	S/16	0	0	297	15S	Cr	0
	S/32	0	0		10S	Cr	0
	S/64	0	0		5S	Cr	0
237	S/1	++++	0		2S	Cr	0
	S/2	++++	0		S/1	Cr	0
	S/4	++++	0		S/5	+++	+
	S/8	++++	+		S/15	+	+
	S/16	++	+		S/50	0	0
	S/32	0	0		S/100	0	0
	S/64	0	0	237	15S	Cr	0
234	S/1	++++	+(f)		10S	Cr	0
	S/2	++++	+		5S	Cr	0
	S/4	++++	+		2S	Cr	0
	S/8	++	+		S/1	Cr	0
	S/16	0	0		S/5	++	+
	S/32	0	0		S/15	+	+
	S/64	0	0		S/50	0	0
					S/100	0	0
				219	15S	Cr	0

Modified Richards' Medium			
	Concentration	Pycnidia	Spores
	10S	Cr	0
	5S	Cr	0
	2S	Cr	0
	S/1	Cr	0
	S/5	0	0
	S/15	+	+
	S/50	0	0
	S/100	0	0

Modified Coons' Medium			
Culture	Dextrose	Pycnidia	Spores
	Per cent		
166	2	Cr	+
	1	Cr	+
	0.5	Cr	+
	0.2	++++	+
	0.1	++++	+
217	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++++	0
	0.1	++++	0
237	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++	0
	0.1	++++	0
247	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++++	+
	0.1	++++	+
342	2	Cr	0
	1	++++	0
	0.5	++++	+
	0.2	++++	+
	0.1	++	+

was used in standard strength and diluted. The results of this experiment also are shown in table 3. This did not seem to be a favorable medium for *Phyllosticta solitaria* in any strength and was not used further.

EXPERIMENTS INVOLVING BOTH QUALITY AND QUANTITY OF FOOD

The above series of experiments had shown that 2 media, S/10 potato-dextrose agar and S/5 bark-decoction agar, favored growth and sporulation of *Phyllosticta solitaria*. Since the composition of such media is unknown, and, at best, must be somewhat variable, it seemed desirable to devise a medium of known composition that could be relied on to induce formation of pycnidia and spores. It was decided to use as a basis for this experimentation a medium found by Coons (4) to be favorable for growth and sporulation of *Plenodomus fuscomaculans*. Since it was thought probable that dextrose would be a favorable source of carbon for *Phyllosticta solitaria*, as it is for fungi in general, and since it is cheaper than maltose, the former sugar was used.

Coons' medium, KNO_3 2 gm., KH_2PO_4 2.7 gm., $MgSO_4$ 1.25 gm., water 1,000 cc., was prepared with the following concentrations of dextrose: 2 per cent, 1 per cent, 0.5 per cent, 0.2 per cent, 0.1 per cent, and each solution was solidified with agar (Table 3). Two of the cultures (166 and 342) used in this experiment were known to sporulate consistently, 2 (217 and 237) were believed to be nonsporulators, and a fifth culture (247) was somewhat variable in performance. The same or a similar set of cultures was used in many of the following experiments.

It is evident from a study of table 3 that *Phyllosticta solitaria* reacts readily to quantity of carbon (supplied as dextrose) in the medium. The behavior of culture 166 is not fully shown in the table, since the quantity of spores produced (number of sporulating pycnidia) in 2 per cent dextrose was very small, and increased as the series descended. It was concluded that a suitable quantity of carbon for the needs of this fungus would be that furnished by a 0.10 per cent concentration of dextrose.

A second experiment in which the salt fraction of Coons' medium was reduced to $\frac{1}{10}$ standard strength and the dextrose concentration varied is reported in table 4. This, again shows the desirability of a low concentration of dextrose and indicates the further possibility that some or all of the salts might be used in lower concentration. Further experiments were, therefore, conducted to learn the most favorable concentrations of the various salts.

The results of an experiment in which the potassium nitrate content of the Coons' medium was varied and dextrose concentration kept at 0.10 per cent are shown in table 5. Sporulation was quite as good when the potassium nitrate was used in a concentration of 0.31 gm. per 1,000 cc. as

TABLE 4.—*Effect of different concentrations of dextrose in S/10 Coons' medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of dextrose	Pycnidia	Spores
	Per cent		
166	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	Cr	+
	0.1	+	0
217	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	Cr	0
	0.1	+	0
237	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	++++	0
	0.1	+	0
247	2	Cr	0
	1	Cr	0
	0.5	Cr	0
	0.2	Cr	0
	0.1	+	+
342	2	++++	0
	1	++++	+
	0.5	++++	+
	0.2	++++	+
	0.1	++++	+

when the medium contained 2.5 gm. per 1,000 cc. There is some indication that a concentration of 5 gm. of potassium nitrate per liter tends to suppress sporulation. Growth differences were not significant, somewhat the best growth being made in the cultures containing 1.25 gm. KNO_3 per 1,000 cc., but good growth occurred in the lowest concentration employed. In view of the general principle that lower food concentrations tend to favor sporulation, a concentration of 0.5 gm. KNO_3 per 1,000 cc. was chosen as convenient and probably favorable.

The next medium was made as follows: KNO_3 0.5 gm., $MgSO_4$ 1.25 gm., dextrose 1 gm., agar 20 gm., water 1,000 cc., and the concentration of KH_2PO_4 was varied. The results of this experiment are shown in table 6. Cultures 217 and 237, which seemed to be consistent nonsporulators, were replaced in this experiment by 2 others, 1 of which (299) was known to produce spores. Culture 166 was originally included, but the series became contaminated.

TABLE 5.—*Effect of different concentrations of KNO_3 in modified Coons' medium (containing 0.1% dextrose) on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of KNO_3 in gm. per liter	Pycnidia	Spores
166	5	++++	0
	2.5	++++	+
	1.25	++++	+
	0.63	++++	+
	0.31	++++	+
217	5	++++	0
	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
237	5	++++	0
	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
247	5	++++	0
	2.5	++++	+
	1.25	++++	0
	0.63	++++	+
	0.31	++++	+
342	5	++++	+
	2.5	++++	+
	1.25	++++	0
	0.63	++++	+
	0.31	++++	+

It appears from a study of this table that concentration of KH_2PO_4 is not so important as that of KNO_3 or of dextrose. It can be varied within rather wide limits without greatly affecting the behavior of the fungus. Since a concentration of 1.25 gm. KH_2PO_4 in 1,000 cc. of medium allowed good growth and sporulation, this concentration was chosen for the new medium. It seemed desirable to keep the concentration of KH_2PO_4 above that of the other salt constituents for two reasons: the demand of fungi for phosphorus is known to be comparatively high and the use of acid phosphate tends to produce an acid reaction in the medium.

It remained to determine the proper amount of $MgSO_4$ for this new medium. The results of an experiment designed to discover this are shown in table 7. The fungus did not seem to react markedly to changes in $MgSO_4$ concentration within the limits of the experiment. In keeping with the plan

TABLE 6.—*Effect of different concentrations of KH_2PO_4 in modified Coons' medium (containing 0.05% KNO_3, 0.1% dextrose) on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of KH_2PO_4 in gm. per liter	Pycnidia	Spores
166	5	++++	0
	(Remainder of series contaminated)		
231	5	++++	0
	2.5	++++	0
	1.25	++	0
	0.63	+	0
	0.31	+	0
247	5	++++	+(f)
	2.5	++++	+
	1.25	++++	0
	0.63	++++	+
	0.31	++++	0
299	5	++++	+
	2.5	++++	+
	1.25	++++	+
	0.63	+++	+
	0.31	+++	+
342	5	++++	+(f)
	2.5	++++	+(f)
	1.25	++++	+
	0.63	++++	+
	0.31	++++	+

to have the total food concentration low, a concentration of 0.75 gm. $MgSO_4$ was chosen as convenient.

The new medium, hereafter referred to as the K.U. medium, is constituted as follows: KNO_3 0.5 gm., KH_2PO_4 1.25 gm., $MgSO_4$ 0.75 gm., dextrose 1 gm., water 1,000 cc. When 2 per cent agar is added this medium allows good growth and sporulation. Its acidity is pH 5.2 to pH 5.4 This, as will be shown later, is a favorable degree of acidity for the fungus in question.

It was, of course, not known whether any one salt was present in this medium in exactly the best amount nor whether the physiological balance between the various constituents was ideally realized. A series of cultures was, therefore, made according to the triangle system well known in plant physiology. The concentration of each salt was varied between limits of 0.25 gm. and 2.00 gm. per 1,000 cc. with intervals of 0.25 gm. between adjacent cultures. Each culture was made in duplicate and 5 sporulating strains of *Phyllosticta solitaria* were used. The results of this experiment

TABLE 7.—*Effect of different concentrations of $MgSO_4$ in modified Coons' medium (containing 0.05% KNO_3, 0.125% KH_2PO_4, 0.1% dextrose) on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Concentration of $MgSO_4$ in gm. per liter	Pycnidia	Spores
166	2.5	++++	+
	1.25	++++	+(f)
	0.63	++++	+(f)
	0.31	+	+
231	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
247	2.5	++++	0
	1.25	++++	0
	0.63	++++	0
	0.31	++++	0
299	2.5	Cr	+
	1.25	++++	+
	0.63	++++	+
	0.31	++++	0
342	2.5	++++	+
	1.25	++++	+
	0.63	++++	+
	0.31	++++	+

showed that best sporulation occurred when the concentrations of the various salts per 1,000 cc. were as follows:

A.	KNO_3	0.50	gm.	KH_2PO_4	1.25	gm.	$MgSO_4$	0.75	gm.
B.	"	0.25	"	"	1.25	"	"	1.00	"
C.	"	0.25	"	"	1.00	"	"	1.25	"
D.	"	0.25	"	"	0.75	"	"	1.50	"
E.	"	0.50	"	"	0.50	"	"	1.50	"

This would indicate that a satisfactory medium for the purpose desired might contain: KNO_3 0.25 to 0.50 gm., KH_2PO_4 0.50 to 1.25 gm., $MgSO_4$ 0.75 to 1.50 gm., water 1,000 cc.

SOURCES OF NITROGEN FOR PHYLLOSTICTA SOLITARIA

Although the K.U. medium described above is entirely satisfactory for growth and sporulation of *Phyllosticta solitaria*, it seemed advisable to investigate other possible sources of nitrogen and carbon.

The first experiments were designed to test the availability of asparagin as a nitrogen source as opposed to potassium nitrate. For this purpose a

medium devised by Brown (2) and intended to approximate the constitution of potato-dextrose agar was used. The salt constituents of this medium are as follows: asparagin 2.0 gm., K_3PO_4 1.25 gm., $MgSO_4$ 0.75 gm. per 1,000 cc. In 1 experiment this medium was used as just given and the dextrose concentration was varied as follows: 2 per cent, 1 per cent, 0.5 per cent, 0.2 per cent, 0.1 per cent. In this series of cultures growth was slow and restricted in amount and, except in the lowest concentration of dextrose, consisted in a thick, piled-up sclerotial crust, such as had been found in previous experiments when excess food was present. Sporulation occurred in all cultures but separate pycnidia formed only in the cultures containing 0.1 per cent dextrose.

TABLE 8.—*Effect of different concentrations of asparagin in Brown's medium (containing 0.1% dextrose) on growth and sporulation of Phyllosticta solitaria*

Culture	Concentration of asparagin in gm. per liter	Pycnidia	Spores
231	2.0	0	0
	1.0	0	0
	0.5	0	0
	0.2	+	0
247	2.0	Cr	0
	1.0	Cr	0
	0.5	+	0
	0.2	+	0
299	2.0	Cr	0
	1.0	Cr	0
	0.5	++++	+
	0.2	++++	+
342	2.0	0	0
	1.0	0	0
	0.5	0	0
	0.2	++++	+

In a second experiment (Table 8) the dextrose concentration was kept at 0.1 per cent and the concentration of asparagin was varied. The suppression of sporulation by excessive concentrations of asparagin is evident. But, even in the lowest concentrations, mycelial growth was restricted. It was concluded that no modification of Brown's medium would be particularly suitable for *Phyllosticta solitaria*.

In a further experiment (table 9) potassium nitrate of Coons' medium was replaced by other nitrogen compounds. The amount of each compound used was such as to supply as much nitrogen (0.28 gm. per liter) as that contained in the 2 gm. per liter of potassium nitrate in the original Coons'

TABLE 9.—*Effect of different sources of nitrogen in modified Coons' medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Source of N	Pycnidia	Spores
166	KNO_3	++++	+
	NH_4NO_3	++++	0
	$(NH_4)_2SO_4$	++++	+
	NH_4Cl	++++	+(f)
	Ammonium acetate	0	0
	" oxalate	No growth	No growth
	Asparagin	++++	0
	Albumen	++++	+
	Peptone	++++	0
231	KNO_3	++++	+
	NH_4NO_3	++++	0
	$(NH_4)_2SO_4$	++++	0
	NH_4Cl	++++	0
	Ammonium acetate	++++	0
	" oxalate	No growth	No growth
	Asparagin	++++	0
	Albumen	++++	0
	Peptone	++++	0
247	KNO_3	++++	+
	NH_4NO_3	0	0
	$(NH_4)_2SO_4$	0	0
	NH_4Cl	0	0
	Ammonium acetate	++++	0
	" oxalate	No growth	No growth
	Asparagin	0	0
	Albumen	++++	0
	Peptone	++++	0
299	KNO_3	++++	+
	NH_4NO_3	++++	0
	$(NH_4)_2SO_4$	++++	0
	NH_4Cl	++++	0
	Ammonium acetate	Cr	+
	" oxalate	No growth	No growth
	Asparagin	++++	+
	Albumen	++++	+
	Peptone	++++	+
342	KNO_3	++++	+
	NH_4NO_3	++++	+(f)
	$(NH_4)_2SO_4$	++++	+
	NH_4Cl	++++	+
	Ammonium acetate	Cr	0
	" oxalate	No growth	No growth
	Asparagin	Cr	0
	Albumen	++++	+
	Peptone	Cr	0

medium. Of the various compounds tried potassium nitrate seemed to be most favorable to sporulation, with albumen next. Asparagin and peptone seemed somewhat less favorable and the other compounds distinctly unfavorable. (In the case of compounds containing both nitrogen and carbon, parallel cultures without dextrose showed that none of these compounds furnished much available carbon for this fungus).

SOURCES OF CARBON FOR PHYLLOSTICTA SOLITARIA

The K.U. medium described above contains dextrose as a source of carbon. Coons (4) found maltose especially favorable for growth and pycnidium formation of *Plenodomus fuscomaculans*. It seemed desirable to compare these and other carbon compounds as to availability for *Phyllosticta solitaria*.

In order to avoid concentration differences Coons used equimolecular solutions of various carbohydrates. It seems that carbon-equivalent solu-

TABLE 10.—*Effect of different sources of carbon in K. U. medium on pycnidium formation and sporulation of Phyllosticta solitaria*

Culture	Source of C	Pycnidia	Spores	Culture	Source of C	Pycnidia	Spores
166	Arabinose	++++	+		Maltose	+	0
	Xylose	Cr	0		Lactose	+++	+
	Rhamnose	Cr	0		Raffinose	++	0
	Dextrose	+	0		Starch	0	0
	Mannose	++++	+(f)		Dextrin	0	0
	Galactose	++++	+	299	Arabinose	++++	0
	Levulose	+	0		Xylose	.....	.....
	Sucrose	++++	+		Rhamnose	+	0
	Maltose	++++	0		Dextrose	++++	+
	Lactose	++	0		Mannose	++++	+
	Raffinose	++	0		Galactose	++	0
	Starch	++	0		Levulose	++++	+
	Dextrin	+	0		Sucrose	++++	+
231	Arabinose	++++	0		Maltose	++++	+
	Xylose	.....	.....		Lactose	++++	+
	Rhamnose	+	0		Raffinose	++++	+
	Dextrose	++++	0		Starch	++++	+
	Mannose	++++	0		Dextrin	+++	+
	Galactose	++	0	342	Arabinose	+	0
	Levulose	++	0		Xylose	.....	.....
	Sucrose	++++	0		Rhamnose	+	+
	Maltose	++++	0		Dextrose	++++	+
	Lactose	++++	0		Mannose	++++	+
	Raffinose	++++	0		Galactose	++++	+
	Starch	++++	0		Levulose	+++	+
	Dextrin	++++	0		Sucrose	+	+
247	Arabinose	Cr	+(f)		Maltose	+	0
	Xylose	.....	.....		Lactose	+	0
	Rhamnose	0	0		Raffinose	+	+
	Dextrose	++++	+		Starch	+	0
	Mannose	++++	+		Dextrin	+	0
	Galactose	+	+				
	Levulose	++++	+				
	Sucrose	++++	+				

tions would be preferable. Since carbohydrates furnish carbon to the fungus, 2 solutions, to be comparable on the basis of food value, should contain equal weights of carbon per unit volume; and, in view of the well-known tolerance by fungi of differences in osmotic concentrations, the molecular concentration should, in fairly dilute solutions, be of secondary importance. For example: in a molecular solution of dextrose there are 72 gm. of carbon per liter; in a molecular solution of sucrose there are 144. In a 1 per cent solution of dextrose there are 40 gm. of carbon per liter, and in a 1 per cent solution of sucrose there are 42. To be strictly comparable, if 1 solution contains 1 per cent dextrose, the other solution should contain 0.95 per cent sucrose. Since the standard for comparison in these studies was a solution containing 0.1 per cent dextrose, sucrose and similar carbon compounds should have been used in a concentration of 0.095 per cent. Practically, the difference between 0.1 per cent and 0.095 per cent solutions would seem to be negligible. The procedure, therefore, was to add the different carbohydrates used in concentrations of 1 gm. per liter to the culture medium: KNO_3 0.5 gm., KH_2PO_4 1.25 gm., $MgSO_4$ 0.75 gm., agar 20 gm., water 1,000 cc.

It is apparent (table 10) that a number of sugars will furnish available carbon for growth and sporulation of *Phyllosticta solitaria*, a notable exception being xylose. There seems to be little choice between dextrose, levulose, mannose, and sucrose. Mycelial growth was somewhat more vigorous on sucrose than on any of the others. Maltose does not seem to be a good sugar for this fungus. Not only did it inhibit sporulation, but it gave rise to a restricted growth of appressed and buried mycelium. Many of the pycnidia also were buried in the medium.

INFLUENCE OF HYDROGEN-ION CONCENTRATION

A series of cultures was prepared in which the initial hydrogen-ion concentration of the K.U. medium was varied by the addition of suitable amounts of lactic acid or of sodium hydroxide. This series ranged from pH 3.6 to pH 7.2, with intervals between each culture of 0.2 pH. Strains 342 and 299 (good sporulators) were used and the whole series was run in duplicate. It was found that abundant formation of pycnidia and good sporulation occurred in all cultures from pH 4.2 to pH 5.8, inclusive. The remainder of the series, from pH 6.0 to pH 7.2, showed in some cases the formation of a sclerotial crust but in no case formation of pycnidia or spores. Mycelial growth of strain 299 was best at pH 5.4, of 342 at pH 5.2. It is to be noted that this is the approximate hydrogen-ion concentration of the normal K.U. medium.

INFLUENCE OF TEMPERATURE

Guba (6) has studied the effect of temperature on the time of appearance of growth and the time necessary for pycnidium (pycnosclerotium)

TABLE 11.—*Effect of temperature on growth, pycnidium formation, and sporulation of Phyllosticta solitaria*

First Experiment—Observations at end of 30 days					Second Experiment—Observations at end of 20 days				
Culture	Temperature	Growth	Pycnidia	Spores	Culture	Temperature	Growth	Pycnidia	Spores
	° C.	Mm.				° C.	Mm.		
166	12–14	2	0	0	231	6–8	0	0	0
	17–19	10	+	0		13–15	0	0	0
	22–24	20	++	0		18–20	20	0	0
	26–28	25	++++	0		23–24	20	+++	0
	31–32	5	+	0		27–29	25	0	0
	36–37	0	0	0		31–33	20	0	0
231	12–14	5	0	0	247	6–8	0	0	0
	17–19	20	++++	0		13–15	10	Cr	0
	22–24	30	+++	0		18–20	20	0	0
	26–28	45	++++	0		23–24	30	0	0
	31–32	40	+	0		27–29	25	0	0
	36–37	0	0	0		31–33	20	++	+
247	12–14	5	0	0	299	6–8	0	0	0
	17–19	15	Cr	0		13–15	8	Cr	0
	22–24	40	+++	+		18–20	15	++++	+
	26–28	45	++++	+		23–24	25	++	+
	31–32	35	++++	0		27–29	35	++++	+
	36–37	0	0	0		31–33	10	0	0
299	12–14	5	0	0	342	6–8	0	0	0
	17–19	20	Cr	0		13–15	5	0	0
	22–24	35	++++	+		18–20	20	+++	0
	26–28	40	++++	+		23–24	25	+++	+
	31–32	20	+++	0		27–29	30	++++	+
	36–37	0	0	0		31–33	10	0	0
342	12–14	5	0	0					
	17–19	15	+++	0					
	22–24	20	++++	+					
	26–28	35	++++	+					
	31–32	10	+	0					
	36–37	0	0	0					

formation by *Phyllosticta solitaria*. He states that the minimum temperature for growth lies between 5° and 10° C., the optimum between 25° and 30° C., and the maximum between 30° and 35° C. Pycnidia were formed at all temperatures at which growth occurred.

In the experiments reported in table 11 the cultures were placed in a battery of temperature chambers modelled after those described by Livingston and Fawcett (11), the cooling being accomplished by means of an electric refrigeration unit.

The results of these experiments indicate that the minimum temperature for growth lies between 8° and 12° C. (Guba found that growth began after 25 to 35 days in cultures held at 10° C.). The maximum temperature for growth seems to lie between 33° and 35° C., and the optimum between 26° and 29° C. The temperature range for sporulation is narrower than that permitting growth. One culture produced spores at 33° C., and 1 at 18° C., but good sporulation of all cultures occurred only between 22° and 29° C. Although not clearly indicated, the optimum temperature for sporulation perhaps coincides with the optimum for growth.

Phyllosticta solitaria seems to have rather high temperature requirements for sporulation in culture. One is tempted to seek in this peculiarity of the fungus an explanation of the geographic distribution of apple blotch. This disease is less destructive in the northern part of its range and is absent from the northern apple-growing regions. It was reported by Thomas (15) as present in New York in 1922, but did not become established. Judging from the temperature experiments reported here, a consecutive period of 480 hours (20 days) at approximately 70° F. will allow sporulation in culture. It would not be safe, however, to argue from this as to the minimum time and temperature required for sporulation in nature. Aside from the fact that the fungus in nature is growing on a different substratum, there is the possibility that the apple twig may have at times a temperature somewhat above that of the surrounding air, as shown by Stevens (14) for twigs of certain plants. Perhaps a study of the temperature requirements of this fungus for spore germination and infection may help to explain the failure of apple blotch to become established northward. It is well, however, to recall in this connection the opinion expressed by Anderson (1), who, from observation of the northward advance of apple blotch in Illinois, feels that it is only a matter of time until the disease becomes serious in northern localities.

OCCURRENCE OF SPORULATING STRAINS

In the early part of this work, isolations were made from various sources and at various times of the year, and the fungus was grown on various media, principally potato agar (without sugar) and potato-dextrose agar. Of these early cultures 141 produced spores at some time or other; 114 did

not. During the latter part of the work 30 cultures were specially tested as to sporulation on S/10 potato-dextrose agar, S/5 bark-decoction agar, Difco corn-meal agar, and K.U. agar. Twenty cultures produced spores on one or more of these media; 10 did not. In several of the experiments reported above there were included sporulating cultures, nonsporulating cultures, and weakly sporulating cultures. It is apparent from a reference to the tables that none of these cultures changed its character under the influence of any of the factors studied. It is quite evident that there are sporulating and nonsporulating strains of *Phyllosticta solitaria*. This fact may possibly account for the failure of other investigators to obtain sporulation in culture, though it is evident that in many cases these investigators were employing media too rich for good sporulation.

Presumably both nonsporulating and sporulating strains might be secured from one parent-sporulating strain, but the only attempt made to do this was unsuccessful. A number of monospore isolations were secured from pycnidia produced in cultures 168, 247, 299, and 342, and the subcultures were tested on K.U. agar. They all produced spores.

SALTATION IN CULTURE

The appearance of light colored sectors devoid of pycnidia in the dark pycnidium-bearing cultures of *Phyllosticta solitaria* has been observed several times during the course of this work. Such sectors have usually arisen in cultures low in food materials or when the nutrients used have not been favorable to the development of the fungus. Transfers from such sectors have yielded light colored, nonsporulating cultures distinct from the parent type. Some of these saltants have preserved their character through several successive transfers to fresh media; others have reverted to the parent type after 1 or 2 transfers. This indicates that non-sporulating strains may arise from sporulating strains by saltation. Occasional loss of sporulating capacity has been observed in stock cultures kept in the laboratory over a period of years. It is thought that this may have come about through the accidental transfer of a nonsporulating saltant. On the other hand, many strains of the fungus have retained their capacity to sporulate after having been kept alive by mycelial transfer more than 6 years.

AUTOLYSIS

It has been observed that there is a favorable period for finding of spores in culture. Pycnidia with spores may generally be found in a culture 10 days or 2 weeks old. Some time later autolysis sets in. Spores seem to be digested first, mycelium later and more gradually. Spores can never be found in very old cultures. The onset of autolysis is, of course, variable and is influenced by such factors as medium and temperature. In general, cultures 2 months old or older may not be expected to show spores.

VIABILITY OF SPORES FROM CULTURE

A number of tests have shown the spores produced in culture to be viable. Spores from culture have been used for inoculating young, potted apple trees growing in the greenhouse. Such inoculations have resulted in production of typical apple-blotch leaf spots.

DISCUSSION

It will be apparent from the results of this study that the procedure for obtaining cultures of *Phyllosticta solitaria* yielding an abundant supply of spores is simple. It consists, first, in making a considerable number of isolations to insure that some of the strains secured will be capable of sporulation, next, in growing the fungus on a suitably dilute medium, such as S/10 potato-dextrose agar, S/5 bark-decoction agar, or the K.U. agar described above.

SUMMARY

The apple-blotch fungus, *Phyllosticta solitaria* has been found to produce pycnidia and spores on a variety of media.

Spore production in culture is not influenced by the locality from which the fungus is obtained, by the part of the host from which the fungus is isolated, nor by the time of year when isolations are made.

The fungus sporulates equally well whether the cultures are kept in darkness or in the light.

Solid media are more favorable to spore production than liquid media.

Quantity of nutrient apparently exercises the greatest influence on spore production in culture. A too rich medium may inhibit sporulation; one that is too lean may not support enough growth to allow sporulation. Potato-dextrose agar of $\frac{1}{10}$ the usual strength and bark-decoction agar of $\frac{1}{5}$ the usual strength have been found favorable to the production of separate fertile pycnidia.

By varying the concentration of the various constituents of Coons' medium a medium favorable to sporulation has been obtained. This medium contains: KNO_3 0.5 gm., KH_2PO_4 1.25 gm., $MgSO_4$ 0.75 gm., dextrose 0.10 gm., agar 20 gm., water 1,000 cc. Its reaction is pH 5.2 to pH 5.4.

The concentrations of the salt constituents are not important within certain limits, and a favorable medium might contain: KNO_3 0.25 to 0.50 gm., KH_2PO_4 0.50 to 1.25 gm., and $MgSO_4$ 0.75 to 1.50 gm., per 1,000 cc.

Of several compounds tested as sources of nitrogen for *P. solitaria*, potassium nitrate proved most favorable to growth and sporulation, albumen seemed next in value, with asparagin and peptone less favorable, and other compounds unfavorable.

As sources of carbon, dextrose, levulose, mannose, and sucrose were found equally valuable. Maltose was found unfavorable to growth and sporulation, and xylose did not support growth.

On the synthetic medium described above the fungus was found to produce fertile pycnidia when the initial hydrogen-ion concentration was between pH 4.2 and pH 5.8.

The following are the cardinal temperatures for growth of *P. solitaria* in culture: minimum between 8° C. and 12° C., optimum between 26° C., and 29° C., maximum between 33° C. and 35° C. The temperature range favorable to sporulation lies between 22° C. and 29° C. It does not seem possible to find in these temperature relations of the fungus any explanation of its present distribution in nature.

When many isolations of *P. solitaria* are made both sporulating and nonsporulating strains are found. It has not been possible to induce sporulation in nonsporulating strains.

Nonsporulating strains arise from sporulating strains by saltation.

Autolysis of mycelium and spores occurs in old cultures of *P. solitaria*.

Spores from culture were found viable and were successfully used in inoculation experiments.

DEPARTMENT OF BOTANY,
UNIVERSITY OF KANSAS.

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GROWTH RELATIONS IN CULTURE OF THE COTTON-ROOT-ROT ORGANISM, PHYMATOTRICHUM OMNIVORUM

ELIZABETH JANE MOORE

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INTRODUCTION

Since infection is an expression of the equilibrium between host and parasite, the selection of resistant varieties must often depend on minute change of this balance. Quantitative inspection of this balance may show new factors or factor intensities that may be selected for building up varietal resistance.

Since no varieties of cotton resistant to root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, have been recognized, this study has been undertaken in an effort to determine the effect of resistance on the growth of the parasite and to record any variability of this effect in the cotton plant.

HISTORICAL

Monocotyledonous plants are considered immune from the attack of Phymatotrichum root rot, although King and Loomis (9), in 1929, reported active growth of the fungus on the tissue of monocots. In 1931, Ezekiel, Taubenhaus, and Fudge (4) reported an investigation of the nutritional requirements of the fungus. In 1932 the same workers (5) reported studies on the growth of this fungus in various plant juices and correlated this growth with the susceptibility and resistance of the plants to the disease. Growth was measured by the dry weight of the hyphae developed in the juices, undiluted and diluted with distilled water or nutrient solution. In nondiluted autoclaved juices of resistant plants the growth was greatly inhibited, while growth in the juices of susceptible plants was abundant. On dilution of the juices of resistant species a good growth was secured. Monocotyledonous plants evidently contain some substances that, when present in sufficiently large concentrations, inhibit the growth of the root-rot fungus. The resistance of the plants is at least partly based on the presence of these materials.

The assumption may safely be made that growth activity varies with the vigor of the fungus and, as a consequence, offers a valuable means of determining the effects of environmental conditions on host infection and subsequent fungus development. Numerous studies have shown that growth in fungus hyphae is apical. Reinhardt, according to Jost (2, pp. 5–6), in 1856, showed that growth takes place largely at the tip of the apical cell, decreasing basally. The studies of Brown (3) showed typical sigmoid

curves for mycelial growth, with an initial period of slow growth, a period in which maximum growth was obtained, and a constant period, declining with the accumulation of metabolic waste products.

Smith (11 and 12) reported detailed studies on the growth of individual hyphae. By following the development of individual hyphae on inverted miniature plates, he showed that only apical growth occurred on the mycelia of *Botrytis cinerea* Pers.; that cells once separated from the apex showed no elongation. In each hypha the rate at which the tip advanced was not constant. In short, hyphae growth was very slow and increased with elongation up to 20 to 30 fold and finally decreased. This increase was considered to result from the increasing septal resistance to the transport of nutrients through the hypha and ultimately to result in branching. Branches show a sequence of growth rates similar to that of the parent hypha and reduce the rate of elongation of the latter. If the curve of the hyphal length was plotted against the time, it was concave to the time axis; but, if the entire hyphal system was considered, a straight line resulted in most cases.

Fawcett (6) showed that fungi exhibit no tendency toward senility if the staling of the media is prevented and that the rate of growth of the entire hyphal system remains constant for considerable periods. Haines (7 and 8), working with *Sporotrichum carnis* Brooks & Hansford, obtained the logarithmic curve typical of hyphal growth and pointed out that linear measurements are true representations of volume growth since, during the logarithmic period, the diameter is constant. With saprophytic Actinomyces the logarithmic curve of growth rates was also typical, the logarithms of the length plotted against the time giving a straight line.

METHOD

Cultures of strains 24 and 39 were secured from J. J. Taubenhaus and cultures grown from sclerotia obtained from the Temple and San Antonio, Texas, experiment stations. The stock cultures were maintained on potato-dextrose agar at 27 to 29° C.

For observation a modified hanging-plate method was followed. Paraffin cells, 2 in. × 3 in., were attached to large microscope slides enclosed by a large cover slip, carrying the agar plate.

A ruled cover slip was sterilized in a Petri dish, and a thin agar plate poured by introducing 1 cc. of the medium by means of a pipette. A wax-pencil line on the cover slip prevented the medium from flowing to the edge. A 4 mm. cube from the peripheral zone of an actively growing culture was used as inoculum. After 12 to 18 hours the inoculum was removed in order to reduce the complexity of the hyphal system. The plate was then inverted over the cell, which had been sterilized by $HgCl_2$ solution and rinsed

in sterile water, and sealed by running a hot spatula around the edge of the cell. On such a plate the fungus will remain in an actively growing condition for weeks. The cells were incubated at 27 to 29° C. and received no illumination except at the time of observation. The mycelia were observed and measured without disturbing the cell.

Measurements of the hyphae were made by means of a Filar micrometer ocular at 2 to 3 hour intervals over varying periods. Both the parent hyphae and the entire hyphal system were recorded, the first measurement being taken as zero. Since elongation is purely apical, measurements were facilitated by use of the reference lines on the cover slips.

A potato-dextrose medium (250 gm. potato steamed 1 hr. in 500 cc. distilled water, 1 per cent dextrose and 2 per cent agar) was used as a stock and also as a standard for growth to compare with that on other media. Decoction media were prepared from various roots by steeping 1 gm. dry ground root in 200 cc. water for 1 hour, filtering, and adding 2 per cent agar. Root decoction was also added to potato dextrose in the same (1:200) ratio. All media were autoclaved at 15 lb. additional pressure for 20 minutes and remelted when desired in an Arnold steamer.

EXPERIMENTAL RESULTS

First experiments were made to establish a standard rate of growth of individual hyphae of Phymatotrichum under given cultural conditions. Potato-dextrose medium was chosen, since it is a favorable one for this fungus. Growth rates were established for strains 24 and 39 and for a culture obtained from a sclerotium. The average growth, calculated from measurements made on 2 to 14 hyphae in each of these experiments, is recorded in table 1 in the columns following the experiment numbers. The averages of all observations made on a given strain are shown in the final horizontal line of each division. These total averages are summarized in figure 1.

It will be seen that strains 24 and 39 maintained a very similar rate of elongation and that the sclerotium culture exhibited a growth rate of a much higher value. When it is remembered that these observations began 12 to 18 hours after the inoculation of the plates and that growth was well under way, the short period of observation is thought to represent the growth rates under favorable cultural conditions. There appears to be little difference in the growth rates of strains 24 and 39; this can be seen not only in figure 1 but also from the final column of averages in table 1, in which both strains show considerable variability and a total average of 0.158 and 0.146 mm. per hour, respectively. Since these averages include readings over a longer period than have been shown in figure 1, no significant differences in the growth rates of these strains appeared during the periods

TABLE 1.—*Growth of individual hyphae and hyphal systems*[a] *of Phymatotrichum omnivorum as grown on potato-dextrose agar. (Averaged from 2 to 14 hyphae. Exception Experiment XIV, 1 hypha measured)*

Experiment	Strain	Average total growth in mm.									Average rate
		Hrs. 6	12	18	24	30	36	42	48	54	
I	24	0.603	1.215	1.818	2.34	3.663	4.95	6.426	7.47	11.88	0.22
II	"	1.08	1.935	2.835	3.726						0.155
III	"	0.486	1.179	2.034	2.448	2.817	3.915	4.41	4.995	5.04	0.090
V	"	1.17	2.646	4.176	5.949						0.247
XV	"	0.405	1.485	2.475	3.402						0.141
XXI	"	0.36[a]	1.54[a]								0.128
XXII	"	0.765[a]	1.35[a]	2.250[a]							0.125
Average	"	0.695	1.621	2.598	3.573	3.24	4.432	5.418	6.23	8.46	0.158
V	39	0.612	1.242	2.331	3.447						0.143
IX	"	0.864	1.899	2.862	3.825	4.608	4.86				0.135
XIV	"	0.918[a]	1.638[a]	2.043[a]	2.448[a]						0.102
XV	"	0.945[a]	1.395[a]	1.575[a]							0.087
XXI	"	0.918[a]	2.988[a]								0.249
XXII	"	0.720[a]	0.990[a]	2.925[a]							0.162
Average	"	0.829	1.629	2.347	3.240						0.146
XX	Sclerotial	2.34[a]	3.627[a]								0.302
XXI	"	3.24[a]	6.714[a]								0.559
XXII	"	2.745[a]	3.905[a]	8.100[a]							0.450
Average	"	2.775	4.748	8.100							0.437

[a] Denotes measurement of entire hyphal system.

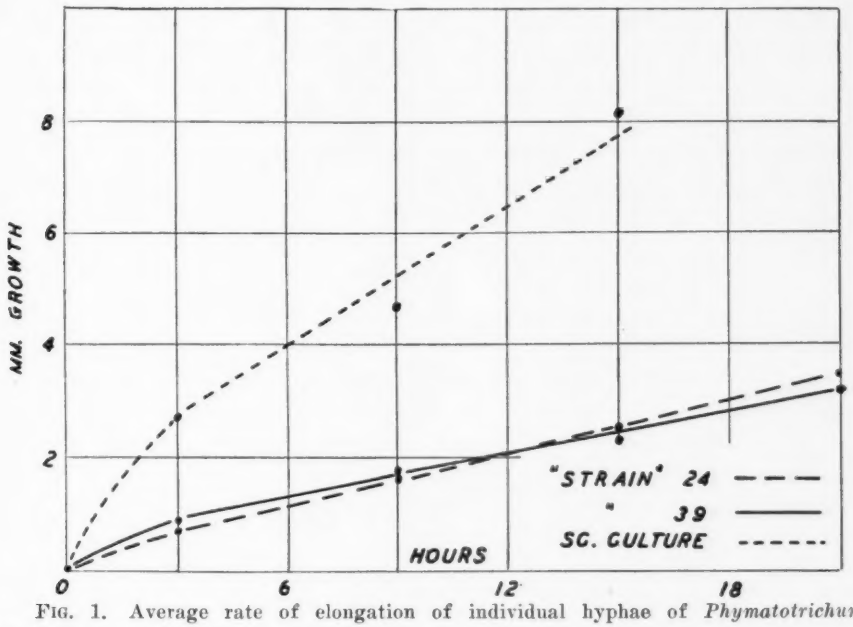

FIG. 1. Average rate of elongation of individual hyphae of *Phymatotrichum omnivorum* on potato-dextrose agar.

of observation. In contrast to this, the sclerotial culture shows an average nearly 3 times that of strains 24 and 39. This culture may be regarded as more vigorous than 24 and 39, although it is more probable that prolonged culture on artificial media of the two strains has resulted in a considerable decrease in vigor.

In order to test the effects of decoctions of dried roots on the growth of Phymatotrichum, a preliminary study was made, using potato dextrose in comparison with a decoction of dried ground cotton root plus 2 per cent agar. Two concentrations of decoction were made, 1:100 and 1:500. Average rates per hour shown by strain 24 were potato dextrose, 0.161; cotton-root decoction 1:100, 0.283; and 1:500 decoction, no growth. For further study a concentration of 1:200 or less was used. The averages obtained from 2 to 12 hyphae are given in table 2. The decoctions were of cotton and corn roots, the former plant being highly susceptible and the latter resistant to this disease in the field (13, 14).

It will be seen that strain 24 in experiments XI and XII showed an average growth rate of 0.099 and 0.157, giving a general average of 0.128. A similar average for a somewhat longer period gave a growth rate of 0.077 on the corn-root-decoction medium. If these rates are compared with the rate on potato dextrose (0.158), it will be seen that neither decoction medium was so favorable for growth and that the growth rate in corn-root-

TABLE 2.—*Growth of individual hyphae of Phymatotrichum omnivorum in root decoctions. (Averaged from 3 to 12 hyphae)*

Experiment	Decoction	Strain	Average total growth in mm. Hrs. 6	12	18	24	30	36	42	48	54	60	66	Average rate
XI	Cotton 1:200	24	0.658	1.152	1.674	2.61	2.97							0.099
XII	" "	24	1.206	2.385	4.698	3.402	4.132	4.599	6.156	7.596				0.157
	Average		0.932	1.768	3.186	3.006	3.551	4.599	6.156	7.596				0.128
XI	Cotton 1:200	39	1.449	2.340	3.150	3.340								0.101
XII	" "	39	1.269	2.700	4.356	6.012	7.497	8.550	11.70	13.59				0.283
	Average		1.359	2.520	3.753	4.976	7.497	8.550	11.70	13.59				0.192
X	Corn 1:250	24	0.869	2.151	3.114	3.213	3.652	4.266	4.266	4.473	4.68	4.89	5.10	0.077
X	" "	39	0.594	1.116	1.494	1.827	2.106	2.637	2.637	3.006	3.34	3.69	4.04	0.061

decoction medium was the lowest. If the growth rates at the end of 24 hours are computed from figures in tables 1 and 2, we have, as values for equal experimental periods, 0.225, 0.133, 0.147 for the cotton-, corn-, and potato-dextrose media, respectively. For this period growth on cotton-decoction medium is above that on potato-dextrose and corn-root decoction, while the difference between the growth on the latter 2 is considerable. Cotton-root-decoction medium is at least as favorable for the growth of strain 24 as potato dextrose and more favorable than corn-root-decoction medium. Computations of growth rates for strain 39 give 0.92, 0.061, and 0.146 for decoctions of cotton and corn roots and potato-dextrose medium. At the 24-hour period the values are 0.194, 0.076, and 0.137, respectively. For strain 39 the average growth rate during the 24-hour period and the longer experimental periods shows the same relationship; in decreasing order, cotton-root decoction, potato-dextrose, and corn-root decoction. The values show somewhat less variability than those obtained for strain 24. Corn-root-decoction medium was relatively less favorable than for the preceding strain, the growth rate on corn-root-decoction medium compared to that on potato dextrose being 89 per cent and 55 per cent for strains 24 and 39, respectively, at the 24-hour period.

From these experiments it is not evident whether the unfavorableness of the corn-root decoction is to be attributed to toxic substances or to a deficiency of nutrients available to the fungus. Succeeding experiments were carried out with freshly made decoctions of a concentration of 1:200. This liquid replaced the water in the usual potato-dextrose formula. Averages of results are given in table 3 and the curves of the values from experiment XIV are shown in figure 2.

It will be seen that corn-root decoction shows consistently the lowest growth rate, and cotton the highest. The average growth rates indicate the same relation, giving values for cotton, potato dextrose, and corn of 0.345, 0.102, and 0.015, respectively. Growth of hyphae on corn-root media developed a very distorted growth characterized by misshapen branches, or all growth ceased and the cells were plasmolyzed.

It is evident that even in the presence of an abundant supply of nutrients, corn-root decoction is an unfavorable medium for the growth of Phymatotrichum and that cotton-root decoction is highly favorable. The growth rate on potato-dextrose-corn-root-decoction medium is but 14 per cent of that on potato-dextrose medium, both media offering the identical nutrient conditions. The fresh corn-root decoction shows a much greater effect than the stored material used in experiment X.

Experiment XV was carried out with media that had been stored for 3 days under sterile conditions before beginning work. Both 24 and 39 were tested in these media and also in a fresh potato-dextrose-Turk's Cap-

TABLE 3.—*Growth of hyphal systems of Phymatotrichum omnivorum on potato-dextrose plus root decoctions*

Experiment	Media		Age of media	Strain	Average total growth in mm.				Average rate
					Hrs. 6	12	18	24	
XIV	Potato dextrose		fresh	39	0.918	1.638	2.043	2.448	0.102
	" "	cotton	"	39	2.790	4.590	6.480	8.280	0.345
	" "	corn	"	39	0.459	0.855	0.855	0.853	0.015
XV	Potato dextrose		3 days	24	0.405	1.485	2.475	3.402	0.141
	" "		3 "	39	0.945	1.395	1.575		0.087
	" "	cotton	3 "	24	0.720	0.945	1.080	1.530	0.063
	" "	"	3 "	39	0.450	0.990	1.395		0.077
	" "	corn	3 "	24	0.630	1.080	1.440	1.620	0.067
	" "	"	3 "	39	0.900	1.170	1.170		0.065
	" "	Turk's Cap	fresh	24	0.360	0.405	0.405		0.022
	" "	" "	"	39	1.440	1.170	1.710		0.095
XXII	Potato dextrose		fresh	24	0.765	1.350	2.250		0.125
	" "		"	39	0.720	0.990	2.925		0.162
	" "		"	Sc. cul.	2.745	3.905	8.10		0.450
	" "	wheat	"	24	0.540	0.540	0.540		0.033
	" "	"	"	39	0.450	0.945	2.205		0.122
	" "	"	"	Sc. cul	1.485	2.250	2.250		0.125
	" "	barley	"	24	0.675	0.990	1.710		0.090
	" "	"	"	39	0.620	3.060	4.680		0.260
	" "	"	"	Sc. cul.	0.720	0.720	0.720		0.036

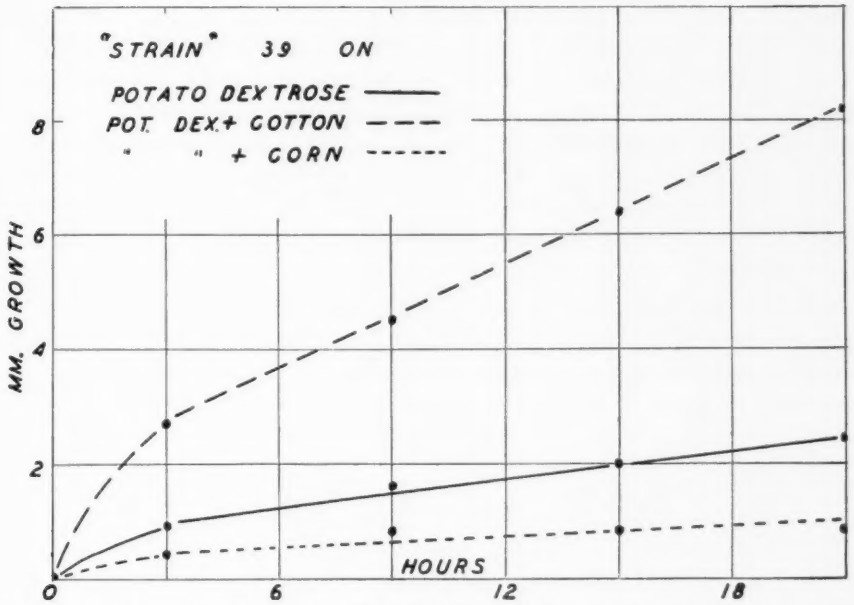

FIG. 2. Average rate of elongation of individual hyphae of "strain" 39 on various media.

root decoction, a plant unique among the Malvaceae in showing a high degree of immunity from root rot (1). In both strains, 24 and 39, the growth in cotton-decoction medium was less than that in potato-dextrose and very similar to that in corn-root decoction. Evidently a 3-day exposure under sterile conditions had largely eliminated the toxic effects of the corn-root decoction as well as the favorable effects of the cotton-root decoction. The 2 strains of Phymatotrichum reacted very differently to the fresh Turk's Cap-root decoction, 39 being unaffected and 24 markedly inhibited.

The same media were checked 3 days later in experiment XVI (not shown in table 3) when the decoctions of cotton and corn roots had been exposed for 6 days under sterile conditions and the decoction of Turk's Cap root 3 days. Strain 39 showed no significant difference in growth on the media, while strain 24 made poor growth on all the media.

The growth rates of strains 24 and 39 and a sclerotial culture were observed on freshly made potato-dextrose, potato-dextrose-barley-root decoction and wheat-root decoction. These monocots, like corn, are resistant to Phymatotrichum. The average values obtained are given in the final column, table 3. As in table 1, the sclerotial culture showed a much greater growth on potato dextrose than either 24 or 39, the average growth rates being 0.45, 0.125, 0.162 for the cultures in the order named above. Wheat-root decoction was markedly unfavorable, the average growth rates for

sclerotial culture and strains 24 and 39 being 0.125, 0.033, 0.122, respectively. The average growth rates in potato-dextrose-wheat-root decoction were 27, 26, and 75 per cent of those obtained in potato-dextrose for the sclerotial culture and strains 24 and 39, respectively. Barley-root decoction with potato dextrose showed a variable result, with the average rate of growth in the order named above being 0.036, 0.09, and 0.261. Sclerotial culture and strain 24 showed but 70 per cent of growth in this decoction compared to that observed on potato-dextrose medium; in contrast to this, strain 39 exhibited a growth on the barley-root decoction nearly twice that observed on potato-dextrose. The hyphae that grew on the decoctions of both wheat and barley roots exhibited the distorted hyphae previously described as those characteristically growing on the corn-root-decoction medium.

DISCUSSION

It will be seen in the final column of table 1 that, although there is considerable variation in the growth rates of a single strain, no trend toward lower values is evident. Thus, the pure cultures that furnished the inoculum for experiment I were maintained by repeated transfers for 6 months and used for experiment XXII. Although the growth rates for strain 24 fell from 0.22 to 0.125, the growth rate of strain 39 rose from 0.143 to 0.162; the lowest observed values for both strains occurring during the progress of this work were followed by high values. Similarly, the sclerotial culture showed no decrease in growth rate during the progress of these experiments. It would seem that during this period there was no decrease in vigor, as shown by the growth rate of any of the cultures.

As measured by growth rate there seems little difference between strains 24 and 39, the values for all experiments being 0.158 and 0.146, respectively. In contrast to this, the sclerotium culture showed a consistently higher rate of elongation. The difference in vigor of growth seems to be inherent in the different cultures, since it appears with different media and at different ages of the cultures. Although it is possibly modified by cultivation, the change is too slow to become evident in the present study. It is to be noted that the greater growth vigor shown by the culture from the sclerotium did not depend on the nutritional value of the sclerotium, since it was present in the culture only when the original isolation was made. Since the observed rates of growth for the sclerotial culture were 2 to 4 times those obtained for either strain, it seems doubtful whether this difference in the growth vigor is sufficient to indicate anything more than degree of activity that could hardly be assumed to have the permanence of a strain. It is evident that this sclerotium culture may be a third and conspicuously active strain, but this seems improbable. On the other hand, the reactions

of the cultures under other nutritional conditions seem to indicate that there is a real physiological difference.

All cultures show a decrease in growth vigor on potato dextrose 4 to 5 days after inoculation. Since, at this time, the fungus colony was not sufficiently large for food to be a limiting factor, it seems apparent that there are present in the medium certain staling products of metabolism produced by the fungus. These may be either carbon dioxide, ammonia, or both; these compounds have an inhibiting effect on the growth of the fungus (10).

In comparison with the growth rates obtained on potato dextrose, decoctions of cotton root proved a favorable medium. By the method of extraction with the dry ground root steeped in water at 100° C. and later autoclaved at 120° C., it does not seem probable that the medium contained an appreciable amount of protein. In the absence of a chemical analysis, it seems probable that the constituents must largely consist of soluble carbohydrates and mineral matter with a small amount of fatty acids and their derivatives and of amino acids. The acceleration of growth on the cotton-root-decoction medium must be due either to the presence of more readily available nutritive materials or to growth-promoting substances. This decoction medium is not favorable after aging, thus indicating that nutrient substances are not involved, since they would hardly disappear or oxidize to toxic substances during such a period.

This view is supported by the results obtained with a medium of potato-dextrose-cotton-root decoction. Growth on such a medium was far above that obtained on potato dextrose alone. This acceleration of growth lasts in such a medium until it has aged for several weeks.

A similar medium containing a decoction from corn roots was very toxic, causing a greatly distorted growth of the filaments and death. After this same medium had aged for several days, the growth rate on it equaled and in some cases excelled that on potato dextrose. This indicates the presence of certain labile water-soluble substances present in the roots of corn that are strongly toxic to the growth of the fungus. These substances in small quantities may act as a stimulant, as appeared in an experiment the results of which are shown in table 3. This is analogous to the stimulating effect of small doses of toxins on animals.

Certain water-soluble toxic materials appear to be present in the roots of Turk's Cap, and their action in causing the death of hyphae in contact with such a medium is similar to that observed in the corn-root-decoction medium. Whether these 2 roots contain similar substances that render them toxic to Phymatotrichum has not been determined, but it seems probable that this may prove to be the case. Decoctions of the same concentra-

tion of roots of barley and wheat had the same toxic effect, although it was not evident to the same degree in all cultures.

Susceptibility and resistance in species tried is paralleled by the growth-promoting and growth-retarding substances closely associated with nutritive values. These substances are more or less labile in solution. Though similar in physiological reaction, they are not identical, as may be inferred from the different degrees of toxicity and permanence in solution. In effective concentrations the mycelia stop growing or develop short, distorted branches and the protoplasm becomes vacuolate and finally becomes plasmolyzed and killed. The reaction of the fungus in the presence of these toxic substances is that of a parasite to unfavorable conditions. The vigor with which the fungus grows in the presence of these toxic substances gives a means of measurement of the effectiveness of the host's resistance to this parasite. The nature of these water-soluble substances present in the roots of resistant plants has not been determined, but it seems probable that they play an important rôle in establishing immunity. Whether the resistance of immune plants is solely due to the growth-inhibiting substances effective in decoctions cannot yet be definitely stated. They seem adequate, at least, to account for the resistant characteristics of monocotyledons.

Thus, it would seem probable that the rate of growth of hyphae of Phymatotrichum on decoctions of roots varies with the resistance of the plant to the parasite. Knowledge of the resistance of various species and varieties to the attack of this organism would be of great economic significance.

SUMMARY

A given culture of *Phymatotrichum omnivorum* grows at a fairly constant rate on potato-dextrose agar.

Different cultures vary in their vigor of growth on potato-dextrose agar.

A recently isolated culture averaged 2 to 4 times the rate maintained by cultures grown on artificial media for long periods.

Extracts from cotton roots furnish sufficient nutrient materials to sustain growth of the fungus. Cotton-root extracts added to potato-dextrose agar greatly accelerate the rate of elongation.

Extracts from corn roots when freshly prepared are strongly toxic to all strains of Phymatotrichum tested. Corn-root extracts in the presence of potato-dextrose agar have a similar effect on the fungus. Such water-soluble substances present in the roots of corn are labile but show a decreased toxic effect when aged.

Small doses of the toxins appear to be growth-stimulating.

Extracts from the roots of Turk's Cap, barley, and wheat all show a similar toxic effect but in varying degrees.

Evidently these water-soluble labile substances present in the roots of plants immune from the attack of Phymatotrichum have some part in establishing this immunity.

DEPARTMENT OF BOTANY AND BACTERIOLOGY,
UNIVERSITY OF TEXAS, AUSTIN, TEXAS.

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A SPHACELOMA ATTACKING NAVEL ORANGE FROM BRAZIL

ANNA E. JENKINS[1]

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INTRODUCTION

The Washington Navel orange (*Citrus sinensis* Osbeck), an introduction from Bahia, Brazil (3, 5, 10, 11, 12), is of great commercial importance in the United States (10, 11). In this country, together with other members of the *C. sinensis* group, it is highly resistant to or immune from attack by citrus scab (9, 14, 15), caused by *Sphaceloma fawcettii* Jenkins.

The present paper reports a disease caused by Sphaceloma, severely affecting the Bahia[2] Navel orange as grown in São Paulo, Brazil, together with studies leading to the identification of the pathogen in this genus.[3]

MATERIAL STUDIED

The specimens on which this study was based, contributed from São Paulo by E. E. Honey, consist of rind of ripe Bahia Navel orange fruits from Sorocaba (Fig. 1, A) collected on July 3, 1931, by F. S. José, and of rind of green fruits collected on Dec. 1, 1931, by C. B. P. Wright, at Limeira (Fig. 1, B), which is said (2) to be the most famous center of citrus production in the State.

NAMES AND IMPORTANCE OF THE DISEASE IN SÃO PAULO

According to E. E. Honey, the disease of the Bahia Navel orange discussed herein is referred to in São Paulo by the Portuguese names "*ver-*

[1] Thanks are due E. E. Honey, formerly of the Escola Superior de Agricultura "Luiz de Querioz," Piracicaba, Estado São Paulo, Brazil, for the specimens on which this study is based. For additional courtesies that have facilitated the work, acknowledgment also is made to others, among them P. H. Rolfs, Escola Superior de Agricultura e Veterinaria, Viçosa, Minas Geraes; H. S. Fawcett, of the Citrus Experiment Station, University of California; P. H. Dorsett, H. R. Fulton, and W. T. Swingle, of the Bureau of Plant Industry, United States Department of Agriculture.

[2] The varietal name "Bahia" as used in this paper or in any reference to the navel orange in Brazil relates to the variety of *Citrus sinensis* that is the original source of the Washington Navel orange of the United States. From plants of the Bahia variety that were introduced into the United States, the late William Saunders, Landscape Gardener and Superintendent of Gardens and Grounds of the United States Department of Agriculture, budded the 2 trees that were introduced into California in 1873 and from which developed (3) what may be termed the "Washington selection" of this variety, now known as the "Washington Navel."

[3] Reference to this paper is made in the U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. **16**: 119. 1932 (Mimeographed).

rucose'' and *''sarna da laranga''* as well as by the English name ''citrus scab.'' In this paper it is referred to as ''navel-orange scab'' (p. 6).

E. E. Honey reports that in São Paulo navel-orange scab affects fruits of the Bahia Navel, often rendering them unmarketable, but that thus far the disease has not been observed on leaves and stems. For example, in an orchard entirely of this variety in which most of the fruit was unmarketable because of the severity of the disease, no trace of it could be found on leaves and stems. Again, as examined in a nursery the leaves of this orange were unaffected by this disease, while the leaves of sour orange, *Citrus aurantium* L., were severely affected by what is possibly the common citrus scab.

CULTURAL STUDIES

Tissue-culture isolations were made from the 2 specimens from Brazil. The Sphaceloma was slower in developing from the ripe fruits and few colonies were produced, but the cultures from the 2 lots of material were, otherwise, of essentially the same characteristics, as exemplified by figure 1, C and D. Compared in parallel cultures on test-tube slants of several different kinds of agar media, with representative cultures of Sphaceloma isolated from Citrus from other sources, the Brazilian fungus was clearly the most distinct of all. It showed a general resemblance, however, to the other cultures.

The 27-day-old cultures on wort (Fig. 1, C, D, L–P) and potato-dextrose (Fig. 1, E, Q–U) agar represent one of these comparisons. Of the wort-agar cultures, C and D are isolations from the Limeira and the Sorocaba specimens, respectively; L and M are from citrus grown in Florida. L (culture 8 A. E. Jenkins) was isolated from rough lemon, *Citrus limonia* Osbeck, in 1916 by H. E. Stevens, and M from Tahiti lime, *C. aurantifolia* Sw., in 1925 by the writer. The Stevens culture was previously employed to illustrate the wrinkled or convolute type of growth in *Sphaceloma fawcettii,* and the culture from Tahiti lime, the smooth or pulvinate type of growth in this fungus (7). In the order named, the isolations shown in N–P are from the following sources: N, from rough lemon grown in New South Wales;[4] O, from calomondin, *C. citis* Blanco, in Formosa,[5] and P, from sour orange, in the Union of South Africa.[6] In the comparison are also included 2 isolations of the Sphaceloma causing scab of avocado, *Persea americana* Mill., formerly confused (6) with *S. fawcettii.* One of these, shown in F on potato-dextrose agar and in H on wort agar, is from avocado grown in Florida and the other, shown in G on potato-dextrose agar and in I on wort agar, from avocado grown in the Union of South Africa.[6]

[4] Culture contributed by F. C. McCleery.

[5] Culture isolated by the writer from a specimen sent by G. Goto.

[6] Culture contributed by E. M. Doidge.

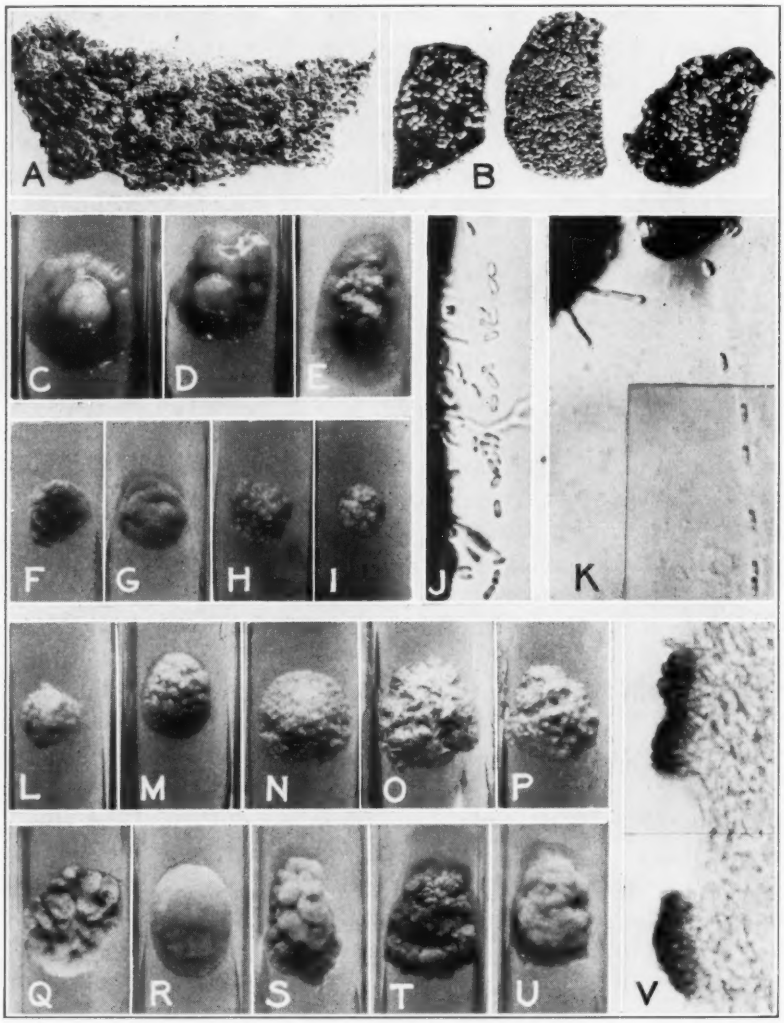

FIG. 1. A and B. Scab on rind of Bahia Navel orange from Sorocaba (A) and from Limeira (B). ×1. C–I, L–U. Comparison of navel-orange scab organism (C–D, on wort agar, and E, on potato-dextrose agar) with 7 other isolations of Sphaceloma, 2 from avocado (F and G, on potato-dextrose agar, and H and I, on wort agar), and 5 from citrus not grown in South America and not including *Citrus sinensis* (L–P, on wort agar, and Q–U, on potato-dextrose agar). All ×1. For further explanation, see text. J–K. Comparison of conidia of *Sphaceloma fawcettii* (J) and of the Sphaceloma causing navel-orange scab (K). Both ×300. V. Acervuli on lesion from specimen shown in B. ×380. V. Photograph by J. F. Brewer; the other photographs by M. L. F. Foubert.

Figure 1, C and D, shows the abundant viscid covering that has thus far been present on wort-agar cultures of the Brazilian Sphaceloma, even when several weeks old. In this same set of wort-agar cultures, observed at the time the photograph was made and a few days later, the 2 cultures from citrus grown in Florida, particularly the one from Tahiti lime (Fig. 1, M), as well as the Australian culture (Fig. 1, N), and one of the avocado cultures (Fig. 1, H), also were covered with a viscid substance. In all cases, however, this was present in a much smaller amount than in the Brazilian cultures. As these cultures aged, those of the Brazilian Sphaceloma became much darker than here represented and were free from the viscid covering. When 7 weeks old they ranged from Buffy Citrine[7] through Buffy Olive to Medal Bronze. The potato-dextrose-agar cultures shown in E and Q–U correspond to those on wort agar shown in D and L–P. On this substrate the Brazilian fungus was distinguished from the other cultures by the Light Orange-Yellow of the outer part of the culture. Later, the Light Grayish Vinaceous central part of this culture became black, while the other isolations remained light colored.

The cultures just described were grown at room temperature. The same isolations were compared in parallel cultures on potato-dextrose-agar test-tube slants at constant temperatures of 0°, 5°, 10°, 20°, and 25° C. For these comparisons, as for those shown in figure 1, the transplantings, from stock cultures, were about 0.5 mm. in diameter. At these several temperatures the Brazilian culture was again separable from all of the other cultures. In all cases essentially the same amount of growth was produced at a given temperature. In month-old cultures there was no growth at 0° C., slight growth at 5° C., and maximum growth at 25° C. During this period none of the viscid substance was observed on any of the cultures, although later a small amount developed about the margins of the cultures from Brazil and Japan.

Figure 1, J, illustrates conidial formation from small mycelial masses of the Stevens culture from Florida, approximately 22 hours after transplanted to corn-meal agar on glass slides. K represents a corresponding culture from the Sorocaba culture. As soon as made, the cultures were placed in Petri dishes lined with moist filter paper. It will be noted that the conidia in the Brazilian culture are of the same size or only slightly smaller than those of the Floridian, but that in the latter culture they are more abundant. In a similar set of equally old cultures, conidia were present in large numbers in both sets of cultures when first examined, but many of those of the Stevens culture had already become swollen or had germinated, while those of the Brazilian culture (Limeira isolation) were

[7] Color readings by J. Marion Shull, based on Ridgway, R., Color Standards and Color Nomenclature, 43 pp., 53 col. pls. Washington, D. C. 1912.

not noticeably so differentiated. Essentially the same conditions obtained when water was substituted for the agar medium.

DISTRIBUTION AND CLASSIFICATION

No definite record of the distribution of the Sphaceloma of the Bahia Navel orange outside of São Paulo is available. At Viçosa, Minas Geraes, as thoroughly examined in May, 1932, by P. H. Rolfs, who sent representative specimens to the writer, this variety was scab-free, while lemon, Rangpur lime, *Citrus aurantifolia,* bittersweet orange, *C. aurantium,* and Satsuma, *C. nobilis unshiu* Sw., were severely diseased.

In the State of Bahia, where it is believed (3) to have "originated as a sport, or mutation, upon a Selecta orange tree," *Citrus sinensis,* the Bahia Navel is apparently free from scab. In the paper just cited, Dorsett, Shamel and Popenoe discuss orange-tree diseases as observed by them while making a study of the Bahia Navel orange in Bahia and elsewhere in southern Brazil in 1913–14. No scab is mentioned, and numerous photographs of navel-orange fruits, including those published in the treatise just cited, are remarkably free from blemishes of any kind. In showing these photographs to the writer recently, Dorsett stated that he does not recall having seen scab lesions on Citrus while he was in Brazil during the period mentioned. Bondar (1, p. 70) has since reported scab in the State of Bahia on seeded oranges but not on the Navel. As of particular interest here, his discussion of the disease in this State, as well as those diseases in the South, is translated from the original as follows:

The disease may be considered as one of the most virulent citrus diseases. In Brazil it is very common in the States of Bahia, Rio de Janeiro, and São Paulo. The species most attacked are those with seeds, and in the first place, sour orange, Selecta, laranja de China (*Citrus sinensis*), lemon, pomelo, etc. The navel is not affected. It is not affected even when grafted on severely diseased sour-orange stocks. For this reason the disease is only of relative importance, since it affects only nurseries. Even so, it should be controlled. In the culture of seeded oranges, such as the Selecta and others, in the States to the South, it is necessary to avoid the propagation of the disease and to eradicate it wherever it appears. On the orange tree, as on the sour orange, it affects leaves, stems and fruits. The fruits lose all commercial value. In the State of Rio de Janeiro, the Minister of Agriculture has prohibited the entrance of any trees of the genus Citrus affected by scab.[8]

[8] The original text from which the translation is made is quoted below:

"A molestia pode considerada como uma das mais virulentas no genero Citrus. No Brasil ella é muito commum nos Estados da Bahia, Rio de Janeiro e S. Paulo. As especies mais atacadas são as de carço, e em primeiro logar a laranja da terra, selecta e

A quarantine measure,[9] dated as early as 1923, recognized the occurrence of scab in the Federal District and the municipality of Nictheroy, in the State of Rio de Janeiro (13).

It would hardly seem that the Sphaceloma of the Bahia Navel orange is identical with that affecting the common sweet orange in Bahia, for we have Bondar's (1) explicit, twice-repeated statement that at Bahia where the common sweet orange is affected by scab the navel is immune. It is possible that the scab of the sweet orange at Bahia is a form of the common citrus scab that has not yet attacked the navel. On the other hand, it is possible that scab of the sweet orange in Argentina, which G. L. Fawcett (4) states is distinct from citrus scab and is practically limited to fruits, may be the same disease as that of the Bahia Navel here reported. This disease has been referred to recently as sweet-orange scab (8).

Because it is highly pathogenic on the Bahia Navel and is culturally distinct from *Sphaceloma fawcettii*, which it resembles, the Brazilian Sphaceloma is classified as a variety of that species, *i.e.*, var. *viscosa*.[10] Additional studies[11] of Sphaceloma pathogenic on sweet orange other than

da China, limoeiro, pomelo, etc. A laranja de umbigo é indemne á esta molestia.'' [p. 70].

''A laranja de umbigo é imune á molestia. Mesmo sendo enxertada nos cavallos da laranja da terra muito atacados, ella não apresenta manifestação alguma deste mal. Devido a este facto a verruga, para a citricultura bahiana, tem uma importancia relativa, prejudicando sómente os viveiros. Nem por isso devemos descuidar de combater o mal.

''Para a cultura de laranja de caroço como selecta e outras, cultivadas nos Estados do Sul é de toda a necessidade evitar-se a propagação do mal e emprehender a extincção onde elle se manifestar. Nas laranjeiras, como na da terra o cogumelo ataca as folhas, os ramos e os fructos. As fructas doentes perdem todo valor commercial. Para o Estado do Rio de Janeiro o Ministerio da Agricultura prohibiu a entrada e a sahida de qualquer arvore do genero Citrus, atacada *por esta molestia.''* [p. 72–73.]

[9] This quarantine measure is quoted below:

RESOLVE, considerar zonos enfestadas pelo fungo Cladosporium citri Mass., doença das laranjeiras, cidreiras, etc., a Districto Federal e o Municipio de Nictheroy, no Estado do Rio de Janeiro, bem como que fiquem nas alludidas zonas, prohibido o transito e a exportação de plantas acima mencionadas, sem o certificado de sanidade, passado pelo encarregado official do Serviço de Vigilancia Sanitaria Vegetal.

Rio de Janeiro, 11 de Abril de 1923—Simões Lopes.

[10] A technical description of this new variety is given as follows:

Sphaceloma fawcettii var. *viscosa* n. var.

Acervuli (Fig. 1, V) on orange rind, dark colored, in section nearly Old Gold, 40 ± μ across, conidiophores 1–4 cells, 6–18 × 4–5 μ, and conidia (only a few seen) 6 × 4 μ. Hyaline conidia produced in culture of same measurements or somewhat larger or smaller. In culture distinguished from the species, particularly by its predominantly Light Orange-Yellow coloration as grown on test-tube slants of potato-dextrose agar; by the persistent viscid substance it produces on this substrate and especially on wort-agar test-tube slants and also by its coloration in old wort-agar test-tube cultures, in 7-week-old cultures, ranging from Buffy Citrine through Buffy Olive to Medal Bronze. In com-

the Bahia Navel in South America will doubtless assist in determining further the relationships of the organism. The term navel-orange scab is proposed to designate the disease caused by *S. fawcettii* var. *viscosa*.

In the United States, Peltier and Frederich (9), discussing the relative susceptibility of Rutaceae to scab in 1923, said that "on the whole . . . the sweet-orange group with an occasional exception, is nonsusceptible to scab." No scab was observed on the Washington Navel orange in Alabama where Peltier and Frederich's (9) studies extending over a considerable period were made. As a result of similar studies in Florida, Winston (14) and Winston, Bowman, and Bach (15) have reported essentially the same conclusions. Winston, Bowman, and Bach (15) say that "the common or sweet orange of commerce . . . is affected so rarely that for all practical purposes it may be considered immune. A trace of scab has been observed a few times on each . . . Florida Seedling, Lue, Maltese, Mediterranean Sweet, Pineapple, and Washington Navel." The fact that the sweet-orange group is essentially immune from scab in southeastern United States is an indication that the sweet-orange disease or diseases just discussed are not distributed there.

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parison with those of the variety, potato-dextrose-agar cultures of the species (culture 8 A. E. Jenkins) were Pale Grayish Olivaceous, and wort-agar cultures, more nearly Buff Pink.

Distribution: On fruits of Bahia Navel orange (*Citrus sinensis* Osbeck), Sorocaba and Limeira, São Paulo, Brazil.

Specimens and cultures examined: On Bahia Navel orange, São Paulo, Brazil, Sorocaba, July 3, 1931, E. S. José, and Limeira, Dec., 1931, Carlos Wright; cultures 345 and 356 A. E. Jenkins, isolated from the Sorocaba and Limeira specimens, respectively. Specimens and representative cultures, the specimen from Limeira designated as the type specimen, have been deposited in the Mycological Collections of the Bureau of Plant Industry, Washington, D. C. A living culture from the Sorocaba specimen (culture 345 A. E. Jenkins) has also been deposited in the Centraalbureau voor Schimmelcultures, Baarn, Holland, where a living culture of culture 8, referred to above, has already been deposited.

[11] Several months after this paper was written, specimens of Sphaceloma on sweet-orange fruits from Argentina were sent by G. L. Fawcett and also on sweet-orange fruits and sour-orange leaves, from São Paulo, by C. P. B. Wright, Director, Serviço de Citricultura, São Paulo. Judging from a preliminary comparison the sour-orange fungus is the same species as *S. fawcettii*. It produces the Cladosporium-like growth characteristic of this species (7). The Sphaceloma on sweet orange from Argentina and São Paulo, as well as that from Paraguay collected by Balansa in 1882 (7, 8), resembles *S. fawcettii* var. *viscosa*. Isolations from the recent specimens of sweet orange resemble cultures of *S. fawcettii* var. *viscosa* more closely than standard culture 8 of *S. fawcettii*, while those of the sour-orange fungus from São Paulo are more nearly like culture 8. Although they resemble each other as just described, all of these cultures are distinguishable from each other. On potato-dextrose agar the Argentine fungus produces Phoma-like structures containing micro-conidia of the same appearance as the conidia shown in figure 1, J and K, except that they are much smaller. A more or less similar stage has been observed for *S. fawcettii* var. *viscosa*, grown on the same substrate.

SUMMARY

On the basis of specimens received from São Paulo in 1931, the existence of a Sphaceloma highly pathogenic on the Bahia Navel orange in São Paulo, Brazil, is reported. The organism is culturally distinct from isolations of Sphaceloma from several kinds of Citrus (not including the *Citrus sinensis* group) grown in other countries, the United States, Japan, Australia, and South Africa, although in its temperature relations it appears to agree with them closely. It is classified as *S. fawcettii* var. *viscosa*. The disease it produces, termed navel-orange scab, appears to be limited to fruits, which it often renders unmarketable.

DIVISION OF MYCOLOGY AND DISEASE SURVEY,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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THE QUINCE-RUST DISEASE CAUSED BY GYMNOSPORANGIUM GERMINALE

H. E. THOMAS

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INTRODUCTION

The morphological aspects and life cycle of the fungus *Gymnosporangium germinale* (Schw.) Kern have been ably treated particularly by Thaxter (17), who demonstrated the connection between the telial and aecial stages, and by Kern (12) in a monograph of the genus and elsewhere. The discovery of the wide prevalence of quince rust on the cultivated apple (9, 11, 14, 18) has pointed to the need for more exact knowledge of many other phases of the disease.

PLANTS AFFECTED

Kern (12) in 1911 recorded the telial stage on *Juniperus communis* L., *J. sibirica* Burgsd., and *J. virginiana*, L., and no additions to the suscepts for this stage seem to have been made.

In addition to the species of Amelanchier, Aronia, Crataegus, Cydonia, and Malus listed by Kern (12) as susceptible, a number of species of Crataegus have been added more recently (1, 13). The writer has collected the fungus on 14 species of Crataegus[1] in central New York, the following 7 of which have not been found in earlier lists: *C. Arnoldiana* Sarg., *C. beata* Sarg., *C. calpodendron* (Ehrh.) Medic., *C. brainerdi* Sarg., *C. filipes* Ashe, *C. holmesiana* Ashe, *C. monogyna* Jacq. The fruits of *C. oxyacantha* L. were rather severely affected by the disease at 2 stations in the outskirts of Ithaca in 1931. Farlow in 1880 (6) recorded *Roestelia aurantiaca* on *C. oxyacantha*. A collection of this fungus on *C. oxyacantha* in the Herbarium of the Department of Plant Pathology of Cornell University was made by H. H. Whetzel, on the University Campus, in 1902.

VARIETAL SUSCEPTIBILITY

While varieties have not been generally recognized in the red cedar, it is a matter of common observation that individual trees may vary rather widely in appearance when grown side by side. It has been observed, furthermore, that different cedar trees equally exposed to inoculation may be quite unequally affected by this disease and, likewise, by the apple and hawthorn rusts caused by *Gymnosporangium juniperi-virginianae* Schw. and *G. globosum* Farlow, respectively (10, 16, 19). This variation in susceptibility is concretely illustrated by the figures in table 1. Two localities

[1] The writer is indebted to Dr. K. M. Wiegand for the identification of the species of Crataegus.

and groups of trees are represented in the table, one by the first 2 trees and the other by the last 3. In each group, the trees are about equally exposed to inoculation by the quince and hawthorn fungi. The nearest source of the apple-rust fungus is somewhat more distant (30 to 40 ft.) for the first group, and no near-by source of this fungus is known for the second group.

TABLE 1.—*Variation in susceptibility of cedar trees to the rusts of apple, hawthorn, and quince*

Tree	Percentage of total infections			Number of infections
	Apple rust	Hawthorn rust	Quince rust	
1	68	32	0	50
2	12	2	86	50
3	0	67	33	200
4	0	7	93	200
5	0	71	29	200

Temporary variations in the susceptibility of trees cannot be invoked as a plausible explanation of the observed facts, since the quince-rust fungus is perennial on the cedar over a long period of years.

Sufficient information is not yet available to permit of a detailed classification of apple varieties as to susceptibility even for a single locality. That a wide variation among varieties does exist, however, is readily apparent. The following figures represent the maximum percentages of fruit infection that have been recorded for several of the more common varieties in Hudson Valley orchards: Delicious 65, Fameuse 21, Hubbardston 28, Jonathan 22, McIntosh 59, Northwestern Greening 24, Winesap 76. Rome Beauty was affected in 41 per cent of the fruits in 1 orchard near Geneva in 1930. In contrast, none of the following varieties has yet been found in the State with as much as 5 per cent of quince-rust infection on the fruit: Baldwin, Ben Davis, Northern Spy, Rhode Island Greening, Twenty Ounce, and Wealthy.

SYMPTOMS

The principal symptoms of the disease on the various affected plants have been described by Weimer (19), Dodge (4), Thomas and Mills (18), and others. A few additional features may be worthy of mention.

On the cedar there is at times a tendency toward the production of small but definite witches' brooms, a characteristic conducive to confusion with the related disease caused by *Gymnosporangium Nidus-avis* Thaxt. Also, on the cedar are found occasionally the galls produced by *G. globosum* partially or largely preempted by *G. germinale* and continuing to produce telia of both species. In such cases the telia of *G. germinale* are usually evident also on the adjacent stems.

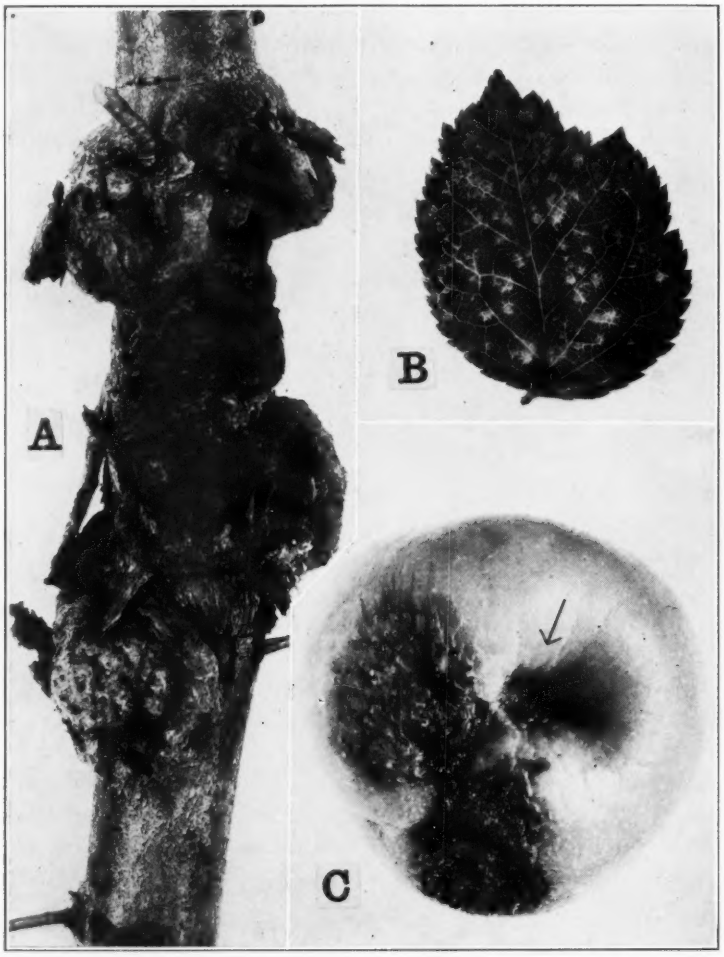

FIG. 1. Symptoms of quince rust. A. Perennial infection on stem of *Crataegus holmesiana*. B. Infection on seedling apple leaves inoculated artifically. C. Necrotic quince-rust lesion (indicated by arrow) and 2 apple-rust lesions on Rome apple fruit.

The disease produced by the aecial stage of the fungus is primarily one of fruits and twigs. On certain susceptible species of Crataegus, the larger stems may be invaded. (Fig. 1, A.). By artificial inoculation, Farlow (7) obtained infection and pycnia on seedling apple leaves (presumably with *Gymnosporangium germinale*) and Miller (15) has had similar results with quince foliage. The writer has obtained heavy infection on leaves of potted plants of *Crataegus* spp. with numerous pycnia, but aecia were produced only sparingly along the larger veins. Chlorotic spots were also produced on seedling apple leaves (Fig. 1, B), but these never developed pycnia and

gradually became less distinct. Similar but still less marked symptoms have been produced on a few inoculated leaves of McIntosh apple.

The symptoms on 24 varieties of apple fruits that have been examined are predominantly necrotic or hypoplastic and rarely hyperplastic. Pycnia when present are individually more prominent than those of the apple-rust fungus but are more widely scattered, rarely, if ever, resulting in the bright orange color that is characteristic of the apple rust. The absence of the orange color, however, is not a reliable diagnostic symptom of quince rust, for apple-rust lesions are sometimes seen even with mature aecia and yet with no visible orange color. In situations favorable for both of these diseases, it is not uncommon to find infections of each on the same fruit, (Fig. 1, C).

ETIOLOGY

The reported differences in susceptibility to apple rust of the same varieties grown in different localities are shown by the work of Bliss (2) to be due in part to distinctly different strains of the fungus. A few infection experiments bearing on the question of pathogenic strains have been made with the quince-rust fungus (all collections from central New York). On 4 occasions, single Crataegus plants were inoculated on opposite halves with telial material from different sources. Abundant infection was obtained in all cases but on 2 plants the later development of the infection seemed more vigorous on one half of the plant than on the other. Again, on 2 different dates, single seedling apple trees were inoculated with spores from one source and later when new leaves had developed the same trees were inoculated with spores from another source. In each instance one of the contrasted inoculations resulted in numerous infections (a maximum of 50 to 90 spots per leaf), while the other was followed by few or none. While these results are not regarded as conclusive evidence of pathogenic strains, it seems probable that more striking differences will be found when collections from more widely separated localities are studied.

An interesting feature in the life history of the fungus has been noted during the past year. In the autumn of 1930, a group of several plants of *Crataegus holmesiana* were found to be rather severely affected by quince rust in the twigs and stems. Several of these were marked and watched during the spring and summer of 1931. Several of the infections persisted through the winter and, judging from appearance and rate of development, some of them probably have survived 2 or more winters. (Fig. 1, A). Dodge (3) has mentioned similar cases of perennation in the aecial stage for *Gymnosporangium Botryapites* (Schw.) Kern and *G. transformans* Ellis.

Germination of Teliospores and Basidiospores.—Weimer (19) states that the germination of teliospores and basidiospores of *Gymnosporangium germinale* is in all respects similar to that described for *G. juniperi-vir-*

ginianae with an optimum temperature (for the teliospores) at 22 to 24° C. In the course of this work several tests have been made mostly at room temperature (about 25° C.) in an effort to learn something of the maximum rate of germination. Under the most favorable conditions that were provided, telia that were immersed in water for 25 minutes and removed to a relatively moist atmosphere, discharged basidiospores in considerable numbers within 2 hours after removal from the water. When dry telia were removed from the substrate, mounted on moist cotton and held at 25° C. there was found after 8 hours an abundant germination of teliospores and the beginning of basidiospore germination. Basidiospores submerged in water at room temperature were observed to develop germ tubes equal in length to the diameter of the spore within 2 hours and 4 to 6 times that length in 10 hours. A similar rate of germination was noted when basidiospores were mounted on a moistened Crataegus leaf and held at 25° C., the germ tubes reaching 5 to 7 diameters in length in 8 hours. It was not determined with certainty whether or not penetration of the leaf had taken place.

Germination of Aeciospores.—Earlier workers experienced difficulty in germinating the aeciospores of *Gymnosporangium germinale* and many other species of the genus. Doran (5) records minimum, optimum, and maximum temperatures for the germination of aeciospores of *G. germinale* at 9°, 16°, and 29° C. The actual percentages of germination are not shown. Thomas and Mills (18) report moderate germination at temperatures of 6° to 21° in spores from fruits previously held at 3°. More recently the writer has obtained up to 49 per cent germination in spores collected November 26, 1930, and kept over night at 3° and a maximum germination of 60 per cent in spores from fallen Crataegus fruits collected on January 22, 1929. However, on March 13, 1929, no viable spores were found in fruits from the same source. On April 4, 1931, a trace to 10 per cent germination was obtained in spores from mummied Crataegus fruits that had remained attached to the tree. These spores, however, were found only in deep-seated aecia with little or no opening to the outside.

In order to test further the influence of previous chilling on the germination of aeciospores, affected quince fruits were placed in open paper bags at 3° and 6° C. on October 1, 1930. Spores from these were set to germinate at 15° on 4 different dates from October 23 to November 13. The averages (each representing 4 counts of 100 spores each) for the spores from the sample held at 3° were 38.7, 28.5, 34.0, and 18.2 per cent, while those for the spores kept at 6° were 22.7, 19.2, 21.0, and 19.5. The relatively small difference between the results from the 2 samples may be due in part to the more rapid drying at the higher temperature.

It was assumed in the earlier tests that precooling was important in inducing the comparatively good germination that was reported. This

appears to have a foundation in the results of a test in which spores were mounted in water and after 25 hours at 6° to 8° C. were moved to 26°. Here a germination of 39 per cent was obtained (200 spores) as compared with 9.5 per cent (800 spores) for spores from the same source placed immediately at 26° without precooling.

However, the best germination in any of the writer's tests was that in spores mounted at 18° C. without precooling. The spores were taken on May 27 and June 20 from a Crataegus plant inoculated in the greenhouse on March 24. Aecia were first seen on this plant on May 23. In these 2 tests a total of 800 spores averaged 68.8 per cent germination with several counts of above 90 per cent. The same number of spores in parallel tests at 26° C. averaged 9.5 per cent germination.

It has been pointed out that the only viable aeciospores found by the writer under natural conditions in late winter were in deep-seated aecia with little or no opening to the outside. It is of interest then to know whether spores that are more exposed to atmospheric influences will survive for any considerable period. With this in mind, aeciospores were mounted on dry glass slides and placed in dry Petri dishes at 3° C. These were removed at intervals and tested for germination at 15° C. The average percentage germination of the spores was 34 at the beginning of the experiment, 18.7 after 2 days, 16.2 after 6 days, and 4.0 after 16 days.

In 2 tests, an attempt was made to detect any influence upon germination due to the presence of cedar leaves, either whole or macerated, in the drops of water. None was found in the 3,600 spores counted. On 2 occasions, May 28, and August 9, 1931, spores were seen (in free-hand sections) to have germinated in droplets of water in the axils of young leaves of potted cedar trees. In the first instance germination was abundant at the end of 25 hours and some evidence was seen of penetration by the germ tubes into the epidermis of the plant. However, this critical point must remain unsettled until a more detailed study is made.

Incubation.—Farlow (7) records the appearance of pycnia on apple-seedling leaves 10 days after inoculation with spores of *Gymnosporangium germinale.* In 4 infection experiments with relatively resistant foliage of apple seedling trees the writer was unable to detect any symptoms until 10 to 18 days after inoculation, and pycnia were never found on these. In contrast in 5 similar tests with susceptible foliage of Crataegus plants, symptoms were seen in 4 to 6 days, and pycnia appeared in 13 to 15 days after inoculation. These plants were grown in a greenhouse in which the temperature is partially controlled at 65 to 70° F.

Relation of Age of Tissues to Infection.—In the spring of 1931, inoculations were made at Ithaca on a few McIntosh fruits on each of 7 dates from May 7 to June 21, beginning when the buds were in the closed-cluster stage. In some cases the moistened telia were suspended over the parts to be inocu-

lated and in others moist cotton was brushed over the germinating teliospores and placed directly on the receptacle of the flowers or the young fruits. Some were covered with celophane bags and others were not. Rain and atmospheric moisture were depended upon for the maintenance of humidity, and these apparently were not adequate, for no infection resulted in any case on the fruit and nothing conclusive was seen on the foliage.

A more successful outcome followed the inoculation of Crataegus leaves in the greenhouse (Table 2). The inoculum was applied copiously to each leaf with a camel's-hair brush and the plants were maintained in a moist atmosphere for 3 days. Infection was so heavy as to preclude the counting of spots. It will be seen, however, that here, as in the case of apple rust on apple foliage (8), the leaf is susceptible in the earlier stages and gradually passes to a condition of complete immunity.

TABLE 2.—*Relation of age of leaf to infection of Crataegus by Gymnosporangium germinale. Records on potted plants 16 days after inoculation*

Branch number	Leaf number (oldest at left)																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Plant 1																									
1	0[a]	0	0	0	0	0	0	0	+[a]	+	+	+	+	+	+	+	+	+	+	+	+	0[b]	0	0	0
2	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0[b]	0	0	0					
3	0	0	0	0	+	+	+	+	+	+	+[b]														
4	0	0	0	0	0	+	+	+	+[b]																
Plant 2																									
1	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+[b]	0	0	0				
2	0	0	0	0	0	+	+	+	+	+	+	+	0[b]												
3	0	0	0	0	0	+	+	+[b]																	

[a] 0 = no infection; + = infection.

[b] Youngest leaf at time of inoculation.

SUMMARY

Plants of the following species of Crataegus not found in earlier lists are reported as affected by quince-rust: *Crataegus arnoldiana, C. beata, C. brainerdi, C. calpodendron, C. filipes, C. holmesiana, C. monogyna.*

Marked variation in the susceptibility of individual cedar trees is shown. Trees susceptible to the quince-rust disease may be relatively resistant to the apple or hawthorn rust.

Basidiospores may be discharged within 2½ hours from the time the telia are moistened. Basidiospores may begin to germinate within 2 hours after they are immersed in water.

Viable aeciospores are found occasionally in nature as late as April (at Ithaca) but in such situations as to render dissemination unlikely.

Good germination of aeciospores is obtained at 15 to 18° without having been preceded by any prolonged rest period.

Symptoms are seen 5 to 8 days after inoculation on foliage of susceptible Crataegus plants. The incubation period in comparatively resistant apple leaves is considerably longer.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

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PHYTOPATHOLOGICAL NOTES

A Destructive Fusarium Wilt of Muskmelons.[1]—In June, 1932, the writer's attention was called to a 6-acre field of muskmelons in Hennepin County, Minnesota, in which more than half of the plants had died within 2 or 3 weeks after emerging from the soil. Many of the hills had been replanted, and at the time of examination the seedlings of the second planting were just emerging from the soil and many of these also were wilting. There were no obvious lesions on some of the wilted plants, although there were definite reddish brown necrotic lesions on the roots and hypocotyl of many of them. On some of the lesions small pinkish spore masses were evident. Microscopic examination revealed these to be spores of a species of Fusarium. Isolations from a considerable number of wilted plants yielded in all cases the same species of Fusarium, which subsequent inoculation experiments proved to be the cause of the disease. When this fungus was compared with a culture of *Fusarium niveum* E. F. S. isolated from wilted watermelons[2] they were found to be very similar in morphological and cultural characters. When grown on several different kinds of media the red to purple pigment produced by the culture from muskmelon was slightly less intense than that produced by the culture from watermelon. The former culture also tended to produce fewer macroconidia and more microconidia than the latter. One hundred tricellular macrospores of each culture produced on potato-dextrose agar were measured. The length of the spores of the muskmelon culture ranged from 24 to 45 μ, while those of the watermelon culture ranged from 27 to 60 μ. There was no appreciable difference in the width of spores of the 2 cultures.

In spite of the close similarity of the 2 fungi in cultural and morphological characters, they differ strikingly in pathogenicity. Watermelons are highly resistant to the culture isolated from wilted muskmelons, while muskmelons are highly resistant to the culture causing wilt of watermelons.

The wilt of muskmelons was found in several fields in which were planted 1 or more rows of watermelons. In these fields more than 95 per cent of the muskmelons were killed by wilt, but not a single watermelon plant was injured. One such field is shown in figure 1, C. These observations were substantiated by greenhouse tests in which Kleckly Sweet watermelons and Golden Osage muskmelons were planted in pots of soil infested with the respective organisms. In these experiments the muskmelons were highly resistant to *Fusarium niveum* and completely susceptible to the culture ob-

[1] Paper No. 1171 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] This culture was kindly furnished by Mr. J. J. Wilson and was isolated from wilted watermelons in 1932 in Conesville, Iowa.

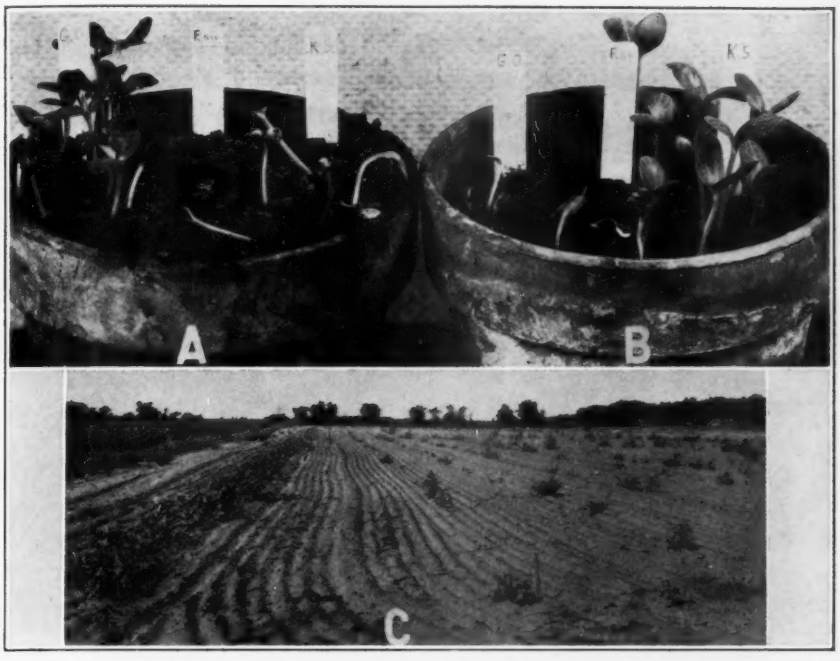

FIG. 1. A and B. Golden Osage muskmelons (G. O.) and Kleckly Sweet watermelons (K. S.) growing in soil infested with *Fusarium niveum* (A) and the Fusarium causing muskmelon wilt (B). C. Field infested with organism causing the wilt shown in B. Note that more than 90 per cent of the muskmelons have been killed by this wilt, whereas none of the watermelons in the single row at the left have yet been killed.

tained from muskmelons. On the other hand, watermelons were highly resistant to the culture from muskmelons and completely susceptible to *F. niveum* (Fig. 1, A and B). These results would indicate that the wilt of muskmelons may be caused by a physiologic form of *F. niveum* or by a closely related species.

A search of the literature has yielded very little definite information about Fusarium wilt of muskmelons. In 1898, Sturgis[3] observed a Fusarium fruiting on the stem of a wilted muskmelon in a field where bacterial wilt was prevalent, and, although no inoculation experiments were made, he suggested that "the wilt disease which has been so prevalent lately in our muskmelon fields may be due in part to the attacks of a species of Fusarium possibly the same as that associated with the wilt of watermelons."

The same year, Selby[4] reported a wilt of garden cucumbers, Japanese climbing cucumber, and muskmelon, and stated that it appeared "to be due

[3] Sturgis, William C. Some common diseases of melons. Conn. Agr. Expt. Sta. Ann. Rpt. 22 (1898): 225–235. 1899.

[4] Selby, A. D. Further studies of cucumber, melon and tomato disease, with experiments. Ohio Agr. Expt. Sta. Bul. 105. 1899.

to the same or to a similar fungus to that causing the southern watermelon wilt described by E. F. Smith." Here also no inoculations were made. Taubenhaus,[5] in 1920, published the results of his investigations of the wilts of watermelons and related crops. In numerous field trials on "watermelon-sick" soil he showed that cantaloupes remained 100 per cent healthy, while watermelons were 69 to 98 per cent killed. Taubenhaus made no mention of a wilt of cantaloupes in this study.

Stone,[6] in 1911, made brief mention of a Fusarium stem rot of cucumbers and muskmelons in the greenhouse but made no inoculations and did not identify the pathogen. It is not probable that he was dealing with the wilt in question, because the greenhouse tests and field observations show that cucumbers are not affected by the wilt of muskmelons occurring in Minnesota.

Cook[7] in 1923, reported a greenhouse disease of melons from which he isolated a Fusarium "very similar to *F. vasinfectum.*" No inoculations were made to determine the pathogenicity of the culture, and the disease is not described in sufficient detail to justify conclusions as to its identity.

Chupp[8] has reported a Fusarium wilt of muskmelon causing a trace to one per cent loss in several fields in New York. This may be the same as the wilt in Minnesota, but it was not described in sufficient detail to justify conclusions.

Although the wilt of muskmelons observed in Minnesota may kill a high percentage of the plants in the seedling stage, plants of any age may be affected. In 2 fields kept under observation additional plants died throughout the season until the occurrence of frost. Plants in later stages of maturity affected with wilt are characterized by brown necrotic streaks beginning at or below the ground line and extending from several inches to 2 ft. or more along 1 side of the stem. On such lesions masses of salmon pink spores may be produced in abundance.

The experience of several melon growers would indicate that the disease has been present in the State for at least 3 years. Records of the Department of Plant Pathology indicate also that it was observed in 1931.

The disease is being studied further and efforts will be made to select wilt-resistant varieties.—J. G. LEACH, University Farm, St. Paul, Minn.

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[5] Taubenhaus, J. J. Wilts of the watermelon and related crops. Texas Agr. Expt. Sta. Bul. 260. 1920.

[6] Stone, G. E. Fusarium disease of cucumbers and other plants. Mass. Agr. Expt. Sta. Ann. Rpt. 23 (Part II): 62–65. 1911.

[7] Cook, Mel. T. A greenhouse disease of melons. Phytopath. 13: 462–463. 1923.

[8] Chupp, Charles. Fusarium wilt of muskmelon. Plant Disease Reporter 14: 160. 1930.

Physiologic Forms of Colletotrichum falcatum Went.—The sudden failure of the sugar-cane variety P.O.J. 213 in Louisiana during the years 1930 and 1931 due to red-rot suggested the activity of physiologic forms of the causal organism (*Colletotrichum falcatum* Went) as a possible explanation. While thus far a detailed study has not completely explained that failure, definite evidence of the existence of physiologic forms has been obtained.

Many hundreds of isolations from rotting cane and leaf midrib lesions in Louisiana, Georgia, and Florida have been compared in culture and in pathogenicity on different varieties of cane. For the latter purpose stalk inoculations have been used. Briefly, the method followed consisted in making 20 inoculations per isolate in uniform material by placing the fungus in cork-borer holes in alternate internodes of the cuttings. These were incubated for 15 days in moist, steamed sawdust at 20–25° C., after which they were split and the spread of the fungus measured. In order to obtain reliable comparisons of large numbers of isolates, it was necessary to express the results mathematically. This was done by calculating the ratios of lateral and longitudinal spread of the lesion to the varying dimensions of the inoculated internodes. Thus, a numerical designation of 1.00 would indicate that the disease had spread the entire length (or width) of the internode, while values of less or more than 1.00, the proportionate parts of the same or adjoining internodes, respectively. By multiplying the ratio for length by that for width a figure is obtained which for convenience in comparing isolates has been termed the "index of virulence."

A representative series of inoculations is presented in table 1 comparing the indices of 4 isolates on three varieties of sugarcane. L–7 was obtained from rotted seed cane of P.O.J. 213 in Louisiana, F–1 from the same variety in Florida, and G–2 from rotted Cayana cane in Georgia. These cultures are representative of large populations isolated from these localities. L–31 was isolated once from severely rotted banked cane of Co. 281 in Louisiana.

The results in the table, which are similar to those from many other tests, show that L–7 and F–1 have approximately the same indices and should be considered as belonging to the same physiologic form. L–31 is more virulent on all three varieties than L–7, F–1 or G–2, its greater virulence on Co. 281 being particularly significant since this variety has been resistant to all other isolates obtained. Because of its greater virulence it has been considered a separate physiologic form, even in the absence of population studies. G–2 is weekly parasitic in comparison with the others and is classed as a third physiologic form.

It has been found that sugarcane varieties possess two kinds of resistance to *Colletotrichum falcatum*: (1) Functional, possessed by those which germinate quickly and are vigorous root formers, thus preventing

TABLE 1.—*Comparative virulence of four isolates of Colletotrichum falcatum on three varieties of sugarcane*

Culture No.	P.O.J. 213				C.P. 807				Co. 281			
	Length	Diameter	Index of virulence	Relative virulence L–7 = 100	Length	Diameter	Index of virulence	Relative virulence L–7 = 100	Length	Diameter	Index of virulence	Relative virulence L–7 = 100
					1st Series							
L–7	1.74	0.97	1.69	100	1.34	0.75	1.00	100	1.01	0.65	0.66	100
F–1	1.98	0.89	1.76	104	1.36	0.69	0.94	94	0.96	0.74	0.71	108
L–31	1.98	1.00	1.98	117	1.41	0.81	1.14	114	1.51	0.91	1.37	208
G–2	1.43	0.87	1.24	73	1.03	0.65	0.67	67	0.64	0.40	0.26	39
					2nd Series							
L–7	1.10	0.68	0.75	100	0.97	0.62	0.60	100	0.54	0.52	0.28	100
F–1	0.94	0.78	0.73	95	0.99	0.70	0.69	115	0.56	0.46	0.26	108
L–31	1.73	0.89	1.54	205	1.31	0.84	1.10	183	0.97	0.73	0.71	254
					3rd Series							
L–7	1.07	0.68	0.73	100	0.97	0.64	0.62	100	0.53	0.40	0.21	100
F–1	0.94	0.74	0.70	96	1.00	0.65	0.65	105	0.56	0.45	0.25	119
L–31	1.73	0.88	1.52	208	1.31	0.45	1.05	169	0.98	0.69	0.68	324

the entrance of the fungus through the root primordia; and (2) physiologic, in which the development of the fungus within the tissues of the stalk is retarded once infection has taken place.—E. V. ABBOTT, Division of Sugar Plant Investigations, Bureau of Plant Industry, Houma, La.

Authority for the Name Cronartium ribicola.—The name of the white-pine blister rust has generally been accredited to Fischer by American authors and to Dietrich by European authors. The writer is clearly responsible for introducing the usage prevalent in America, and possibly for the one exception to the usage in Europe, occuring in Grove's British Rust Fungi.[1]

In the North American Flora, vol. 7,[2] the name was given as Fischer von Waldheim. Not long after the publication of that part of the Flora in 1907 Perley Spaulding visited the herbarium at La Fayette and desired to know the grounds for this selection of authority. It was recognized at the time that Dietrich's name of 1856 (or possibly 1854) was a *nomen nudum*, while Fischer[3] in 1872 had published a description of *Cronartium ribicola* Fischer, nov. sp., and also issued a collection under this name in Rabenhorst's Fungi Europaei Exsiccati, No. 1595. This collection, as represented in the Farlow Herbarium, shows that Fischer had the right fungus, for it bears both urediospores and teliospores. This fact does not, however, affect the nomenclature. The full name of this "Fischer" was not stated at the time, and it remains unknown up to the present, even after much effort expended by the writer and others. The name of Fischer von Waldheim was familiar to American mycologists from the publication of a translation of his extensive paper on the "Biology and history of the development of the Ustilagineae," and it was easy but indefensible to assume the identity of the two Fischers. Although the error was at once recognized it was not convenient to make a correction until the 10th part of the Flora[4] was reached in 1925.

Perley Spaulding[5] has called attention to the unsatisfactory usage by American authors and indorses the conclusion reached by Klebahn[6] in his extended study of the matter in which all the data known up to the present time are carefully considered.

[1] Grove, W. B. The British fungi. 412 pp. University Press, Cambridge. 1913. (p. 316.)

[2] Arthur, J. C. North American Flora **7**: 122. 1907.

[3] Fischer. *Cronartium ribicola* Fischer nov. sp. Hedwigia **11**: 182. 1872.

[4] Arthur, J. C. *L. c.*, p. 692. 1925.

[5] Spaulding, Perley. Dietrich preferable authority for *Cronartium ribicola*. Phytopath. **23**: 203–204. 1933.

[6] Klebahn, H. Der Autor des *Cronartium ribicola*. Ztschr. Pflanzenkr. **41**: 209–213. 1931.

The question to be settled is seemingly very simple. Should the name be written *Cronartium ribicola* Dietrich or *Cronartium ribicola* Fischer, as these are the only authors who have ever referred the fungus to the genus Cronartium? The undisputed facts are these: Dietrich used the name in a local flora, published no description, claimed that it was a new species, and issued in a published exsiccati a specimen that bore teliospores. Fischer published a description, called it a new species, and also issued a specimen in a published exsiccati. In accordance with the International Rules of Nomenclature it must be admitted that Dietrich's name in these respects was by itself, and still is, a *nomen nudum,* while Fischer's name was duly established. One cannot, therefore, agree with the conclusion reached by Klebahn, following an extended and ingenious argument, that: "One thus leaves to Dietrich the honor of being the author of the fungus, since he discovered it, correctly named it and distinguished it, as well as is generally possible without knowledge of the aecidia, as a Cronartium which lived on Ribes." If this procedure were generally adopted, a *nomen nudum* would no longer be a barrier to the acceptance of a name as valid.

But this is not the whole story. Neither Dietrich nor Fischer originated the specific name *ribicola,* but a much earlier author, G. W. Lasch, who published the name in 1842 under the genus Uredo, with a description and specimen in Klotzsch's Herb. Myc. 490. This name, *Uredo ribicola* Lasch, according to Klebahn, cannot be considered in priority, because "teleutospores were not observed." The description was reprinted in Rabenhorst's Deutschlands Kryptogamen Flora,[7] without reference to teleutospores.

The validity of uredosporic names depends at present upon the wording of Rule 49 *bis,* which says in a rather ambiguous and illogical way that a name to be accepted must apply to the "perfect states," that is, the one "which ends in the teleutospore or its equivalent." The Rule has recently been subjected to analysis and interpretation by Faull,[8] who reaches the conclusion that, excluding the (one) example appended to the Rule, "one might very well interpret the 'state' indicated as referring to the entire diploid state, a state in the rusts that produces both uredospores and teliospores." This appears to the writer convincing and acceptable.

Returning to the name for the white-pine blister rust and recognizing Uredo names as in good standing in priority, the correct designation should be *Cronartium ribicola* (Lasch) Dietr. The name was first correctly established by Lasch under the genus Uredo and was subsequently transferred

[7] Rabenhorst, L. Deutschlands Kryptogamen Flora. Erster Band. Pilze. 613 pp. Eduard Kummer, Leipzig. 1844. (p. 579).

[8] Faull, J. H. Taxonomy and distribution of the genus Milesia. Contrib. Arnold Arboretum Harvard University, no. 2. 138 pp. Jamaica Plain, Mass. 1932. (pp. 9–11).

to the genus Cronartium by Dietrich, all in accordance with the accepted rules of nomenclature. This presentation of the subject has been read by Perley Spaulding, who states in his letter of March 8, 1933, that "I am disposed to accept your decision in the matter."—J. C. ARTHUR, Purdue University, La Fayette, Indiana.

Some Promising Fungicides.—At the Arlington Farm, Rosslyn, Virginia, station of the Bureau of Plant Industry, some promising spray materials have been developed that may be of value in the control of plant diseases and for which patent applications have been filed under the provisions of the Act of March 3, 1883, (22 Stat. 625). Because copper compounds appear to be effective in the control of fungous parasites of plants, the experiments have been principally concerned with the improvement of Bordeaux mixture and with the use of very insoluble copper compounds.

These copper compounds, when ground finely enough to pass through a 300-mesh screen or to the standard of Portland cement and mixed with milk of lime, form suspensions that lack the spreading and sticking qualities of Bordeaux mixture. To supply these qualities, an adjuvant, made by combining lime with bentonite, a natural clay-like material forming a gel with water, was developed. The gel-forming property of bentonite is not useful in the spraying of plants, since the material in this form is easily washed off the sprayed plants; but, when lime, slaked or hydrated, is added to a true bentonite, a flocculent precipitate is formed that has excellent spreading and adhering properties and furnishes a highly absorptive surface for the insoluble materials.

The copper compounds that show promise, when finely ground and combined with bentonite flocculated by lime, are: copper oxide, copper silicate, and copper phosphate. These materials are not, at the moment, manufactured for wide-scale commercial use but for experimental purposes can be bought in small quantities from the chemical-laboratory supply houses. A chemical firm has manufactured these materials for our experimental work at a considerable reduction in price over catalogue figures, and we feel sure that these compounds can be made available at very reasonable figures, should they develop into successful orchard sprays.

In a limited way we have used the above materials on Hiley peach and on York and Grimes apples. On peach, copper oxide and copper silicate controlled scab and brown rot quite well without causing injury to the fruit, foliage, or wood. Copper phosphate, although not so injurious as Bordeaux mixture, caused enough injury to peach to make its usefulness on this host doubtful. On apple copper oxide, copper silicate, and copper phosphate were not injurious in any way. Since there was practically no scab in the apple orchard no data on scab control were obtained.

In repeated laboratory tests these fungicides were toxic to the spores of *Glomerella cingulata* (Atk.) S. and S. (bitter rot of apple), *Sclerotinia fructicola* (Wint.) Rehm (peach brown rot), *Cladosporium carpophilum* Thüm. (peach scab), *Venturia inaequalis* (Cke.) Aderh. (apple scab), and *C. effusum* (Wint.) Dem. (pecan scab). The materials exerted their toxicity under the weathering conditions of the orchard environment for at least 10 days and in the case of copper phosphate as long as 21 days. This was determined by placing spores of the test fungi on the slides that had been hung among the leaves of the host and sprayed at the time the host was sprayed. A group of sprayed slides was brought into the laboratory each day and tests were run with standardized spore suspensions. In this manner the longevity of the toxic and inhibitory effect of the fungicides was determined. Bordeaux mixture was always used in comparison and usually other standard materials, such as zinc-lime, lime-sulphur solution, wettable sulphur, and proprietary sulphurs.

The liquid sprays that we used had this general formula:

Copper silicate, copper oxide, or copper phosphate (as the case may be)	2.0 lb.
Hydrated lime or equivalent CaO	4.0 "
Bentonite (one showing a high degree of water-absorbing capacity)	2.0 "
Insecticide (arsenate of lead, etc.)	1.0 "
Water	50.0 gal.

If hydrated lime is used, all the materials may be weighed together, thoroughly mixed, and added to the tank of water in a dry condition. If stone lime is used, this material must be slaked separately and added to the tank, first, in the required amount of water. Then the other ingredients, mixed together dry, may be added. In both cases the tank agitator should be in motion throughout the mixing.

We have found that a dust with good physical properties may be prepared by adding 2 lb. of the copper compound to 4 lb. of hydrated lime and 4 lb. of bentonite. To this may be added 1 lb. of an insecticide such as lead arsenate.—M. C. GOLDSWORTHY and E. L. GREEN, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Mosaic Diseases Affecting Different Legumes in Relation to Beans and Peas.—The viruses causing the mosaic diseases of white sweet clover (*Melilotus alba*), yellow sweet clover (*M. officinalis*), white clover (*Trifolium repens*), alfalfa (*Medicago sativum*), pea (*Pisum sativum*), and sweet pea (*Lathyrus odoratus*) are all transmissible to beans (*Phaseolus vulgaris*), while the virus producing the mosaic disease of red clover (*T.*

pratense) is not. Although a careful examination of the symptoms produced on beans by the viruses from these legumes reveals specific differences, the viruses from white clover and white sweet clover when inoculated into beans produce symptoms, some of which resemble the typical mottled and chlorotic condition of the common bean mosaic.

It is entirely probable that there may be present in the several legumes various other mosaic viruses that may react differently from those that are herein reported, notwithstanding the production of symptomological similarity.

The infection produced by all of the legume-mosaic viruses on beans is systemic with the exception of that of alfalfa, which produces only local lesions. The white-clover-mosaic virus, besides being systemic, may sometimes produce local lesions.

It has not been definitely proved that the viroses of the several legumes (white sweet clover, white clover, alfalfa, pea, and sweet pea) are seed-borne, but preliminary evidence indicates that the mosaics of pea and white sweet clover may be carried in the seed in small percentages. The virus of none of the legume mosaics, except bean mosaic, has thus far been proved to be transmitted through bean seed.

Most bean varieties are susceptible to the viruses of the legumes mentioned. The alfalfa-mosaic virus infected 24 out of 28 varieties tested, and that of white sweet clover 21 out of 22. The virus of white-clover mosaic produced typical symptoms on 24 out of 26 varieties. Thirty-two varieties of beans were tested with the mosaic virus of pea and 31 were susceptible. In the latter case the inoculated plants of many of the very susceptible varieties died.

The effect of the virus of the various legumes on bean varieties that are resistant to their own mosaic, differs, depending on the virus used. The 3 varieties of beans resistant to bean mosaic (Robust, a strain of Great Northern, and a Stringless Green Refugee selection) also are resistant to the alfalfa mosaic. The mosaic viruses of white sweet clover and white clover infect only the Stringless Green Refugee selection, while the virus of pea mosaic infects both this selection and the Robust variety. Only the strain of Great Northern appears to be resistant to all of the viruses used.

In addition to these virus diseases, a mosaic virus (designated as No. III) was isolated from a bean hybrid, whose extract reacts similarly to the legume-mosaic viruses, especially with respect to the susceptibility of certain bean varieties. It differs, however, from the common bean mosaic. The virus is systemic and does not appear to be seed-borne. Twenty-six bean varieties were inoculated with this virus and 24 were susceptible. Of the three varieties resistant to the common bean-mosaic virus, No. III was infectious only to the Stringless Green Refugee selection.

Peas and sweet peas are affected differently from beans when inoculated with the viruses of the various legume mosaics. The red-clover-mosaic virus, inoculated on the pea, produced symptoms somewhat similar to pea mosaic but gave negative results when inoculated on the few varieties of sweet pea tested. Peas and sweet peas were not infected by the alfalfa- and bean-mosaic viruses. The mosaic viruses of white sweet clover and white clover produce destructive symptoms on peas and sweet peas very unlike pea mosaic. The symptoms resemble a streak, in some cases causing death to the sweet pea. The vascular bundles of infected pea and sweet pea roots and stems are discolored and fungi are readily isolated, but in no case have they been proved pathogenic. It is likely that infected plants become weakened by the virus and that saprophytic fungi enter the root system, producing a vascular discoloration.

It is possible that both beans and peas may become infected from the various legume-mosaic viruses in the field as a result of aphid transmission. —W. J. ZAUMEYER and B. L. WADE, Division of Horticultural Crops and Diseases, U. S. Department of Agriculture.

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Scientists from abroad, numbering some thirty-five, will be honored by a banquet at the Hotel Stevens on Thursday evening, June 22.

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